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HEAT INACTIVATION OF LEAF-ROLL VIRUS IN POTATO TUBERS

BY B. KASSANIS

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When potato tubers were stored at 37·5° C. in a humid atmosphere, some lost their germinating power after 20 days but others survived up to 40 days. All tubers infected with leaf-roll virus that survived 25 days at this temperature produced healthy plants. Similar treatments up to 40 days did not free tubers from potato viruses *X* and *Y*.

Except for *X*-disease of peach (Stoddard, 1942) the only successful therapeutic treatments of virus-infected plants have been by the use of heat. Sugar-cane was freed from sereh and chlorotic streak viruses by immersing cuttings from infected plants in a water-bath at 52° C. for 30 and 20 min. respectively (Houtman, 1925; Martin, 1930). Peach trees have been cured of yellows, little peach, red suture and rosette, and periwinkle has been freed from aster yellows virus, by placing infected plants in a room at a temperature between 34 and 42° C. for various periods of time (Kunkel, 1936, 1941). Attempts have been previously made to free potato tubers from leaf-roll and potato viruses *X* and *Y* but they were unsuccessful (Atanasoff, 1922; Johnson, 1922; Blodgett, 1923). Kunkel (1943), however, has found that potato tubers were freed from witches'-broom virus by heating at 36° C. for 6 days. This paper describes a method whereby healthy plants can be obtained from tubers infected with the leaf-roll virus.

EXPERIMENTAL

Experiments were first made by immersing potato tubers for a day in water held at 38–40° C. but this gave little promise of success. Most tubers were killed and the few that germinated produced diseased plants. Tubers in an incubator at the same temperature, however, survived several days' heating, and the proportion of tubers that survived prolonged treatments was increased by raising the humidity of the air This was done by placing a flat open dish of water in an incubator of 6 cu.ft. capacity. The temperature within the incubator was kept constant at 37·5° C.

Dormant tubers were used as the heating killed already developed sprouts. Losses of tubers occurred from a variety of causes, of which black-heart was the most common; a few were lost from an undiagnosed dry rot, and some that appeared to be uninjured by the heating nevertheless did not germinate. Black-heart was particularly prevalent with tubers harvested from field-grown plants, and to avoid this trouble most experiments were made with tubers from infected plants grown in the glasshouse. The tubers weighed from 5 to 30 g.; within this range the larger tubers

survived heat treatment for longer periods than small ones, but the virus was inactivated as soon in large as in small tubers. The variety Majestic was mainly used but tests were also made with Arran Consul and Arran Victory. The potatoes from each infected parent plant were indexed and one was kept as an untreated control; all these gave rise to typical leaf-roll plants. Batches of tubers were removed from the incubator at 5-day intervals and stored until sprouts developed. The heating delayed sprouting and when tubers were planted immediately after treatment a greater proportion rotted than if they were first placed in chitting trays to sprout.

Experiments made during the last 5 years showed that potato tubers can withstand 40 days or more at 37° C., but that the proportion of survivors becomes progressively smaller after 20 days. All tubers that survived this treatment for more than 25 days produced healthy plants. Some were freed from the virus by 10 days' treatment and the proportion of cures increased with time of treatment from 10 to 25 days. Table 1 gives the result of one experiment.

TABLE 1. *Inactivation of potato leaf-roll virus in infected tubers maintained at* 37·5° C.

	Majestic		Arran Consul	
Length of treatment in days	No. of tubers that germinated (25 treated)	No. of tubers that produced healthy plants	No. of tubers that germinated (5 treated)	No. of tubers that produced healthy plants
10	25	1	5	0
15	25	7	5	2
20	18	14	5	4
25	16	16	2	2
30	10	10	0	—
35	10	10	—	—
40	4	4	—	—
45	3	3	—	—
50	0	—	—	—

Plants from the heated tubers have been grown through the whole season without showing symptoms, and tubers harvested from them have themselves produced normal-looking plants in succeeding years. The plants are not symptomless because they are infected with a masked strain, for when some were grafted with scions from leaf-roll plants they developed typical symptoms.

The Majestic tubers used in the experiment recorded were infected with virux *X* in addition to leaf-roll virus. All the treated tubers, even those that survived 45 days' heating, gave plants still infected with this virus. In other similar experiments, heat treatment also failed to eliminate either potato virus *X* or potato virus *Y* from tubers.

Little is known about the properties of viruses which have been inactivated *in vivo* by heat treatments, for none of them is mechanically transmitted to plants. Aster yellows virus has been inoculated to its insect-vector, and Black (1943), studying its properties in crushed viruliferous leaf hoppers, found that it is inactivated by heating

for 10 min. at a temperature between 35 and 40° C. Probably one of the requirements for successful heat inactivation of viruses in living plants is such a low thermal inactivation point. Some viruses with low thermal coefficients, however, can be inactivated by heating infective sap for a few hours at temperatures much below their thermal inactivation points (Bawden, 1943). Tomato bushy stunt and tobacco necrosis viruses, for example, have thermal inactivation points about 80° C., but are inactivated *in vitro* by heating for about a day at 50° C. Leaf-roll virus may be in this group.

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STUDIES ON TWO APHID-TRANSMITTED VIRUSES OF LEGUMINOUS CROPS

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Pea mosaic virus was transmitted by *Myzus persicae* Sulz., *Macrosiphum pisi* Kalt., *M. solanifolii* Ash. and *Aphis fabae* Scop., but not by *Hyperomyzus staphyleae* Koch. It is a 'non-persistent' virus (Watson & Roberts, 1939), and is most readily transmitted when vectors are fasted and then given a short infection feeding. Vector efficiency was not increased by increases in preliminary fasting beyond 15 min. or with increasing infection feeding beyond 1 hr. Most aphids became non-infective within 15 min. when feeding, but fasting aphids remained infective for 3 hr. Species that fed readily on the infected plants were less efficient vectors than those which did not. Seed set by infected plants produced healthy seedlings.

Pea enation mosaic virus persisted in *Myzus persicae* and *Macrosiphum pisi* for more than 140 hr.; its transmission was unaffected by preliminary treatments of aphids. No transmission was obtained until at least 4 hr. after aphids had left infected plants; usually the 'latent' period exceeded 1 day and its duration was apparently unaffected by the length of the infection feeding.

Introduction

The two viruses used came from plants found naturally infected in the open. Their identity is therefore uncertain, but they seem to resemble those given the names of pea mosaic and pea enation mosaic viruses in the list published by the *Review of Applied Mycology* (1946), and will be referred to under those names. The main aim was to study the factors affecting their transmission by aphid vectors, and in order to relate them with viruses described by other workers some of their general properties and their host ranges were also determined.

Techniques used in the insect-transmission studies were the same as those described by Watson (1936, 1938). The terms used in connexion with the aphid-transmission experiments are as follows:

'Infection feeding': the act of feeding by the aphid on the infected plants.

'Test feeding': the act of feeding by the aphid on the healthy test plants after infection feeding.

'Preliminary fasting': fasting of the aphid prior to infection feeding.

'Post-infection fasting': fasting of the aphid immediately after infection feeding.

Pea mosaic virus

Symptoms and host range

Symptoms appeared in pea (*Pisum sativum*), broad bean (*Vicia faba*) and sweet pea (*Lathyrus odoratus*) 5–10 days after infection: in pea the leaf and stipule nearest to

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the point of infection showed vein clearing, which faded after 5–6 days and was replaced by a mosaic, which was the only symptom on leaves and stipules that developed later. Symptoms in broad bean resembled those in pea, but in sweet peas the mottle was very mild and sometimes almost imperceptible. Young plants of subterranean clover were sometimes killed, but in all other susceptible hosts tested the disease was mild.

The virus was transmitted by mechanical inoculation, as well as by aphids, to pea, broad bean, sweet pea, spring vetch (*Vicia sativa*), subterranean clover (*Trifolium subterranean*) and crimson clover (*T. incarnatum*). Plants which were found not to be susceptible to pea mosaic virus were tobacco (*Nicotiana tabacum*) var. White Burley, *N. glutinosa*, French bean (*Phaseolus vulgaris*) vars. Prince and Bountiful, soy-bean (*Soja max*), tomato (*Lycopersicum esculentum*), red clover (*Trifolium pratense*), white clover (*T. repens*), alfalfa (*Medicago sativa*) and lupin (*Lupinus polyphyllus*) var. Russell.

General properties

The properties of the virus were studied using crude sap from infected broad bean leaves as inoculum and the test plants, broad bean seedlings, were dusted with 400-mesh carborundum before rubbing.

The dilution end-point of the pea mosaic virus was between $1/10^4$ and $1/10^5$. The percentage of inoculated plants that became infected at dilutions 1/1, 1/10, $1/10^2$, $1/10^3$, $1/10^4$ and $1/10^5$ were 73, 45, 40, 24, 16 and 0 respectively. The failure to obtain 100 % transmission with undiluted sap, though some plants became infected at $1/10^4$, suggests that there may be some inhibitory substance in the sap of broad bean leaves, the effect of which is reduced by dilution. The thermal inactivation point of the virus when heated for 10 min. was between 60 and 65° C. Sap was still infective after storage at room temperature (maximum 20·4° C. and minimum 17° C.) for 72 hr. but not after 96 hr.

Some viruses of leguminous plants are seed transmitted, but of 1380 peas (var. Lincoln) from infected parents sown under glass, 1365 germinated and none showed symptoms during an observation period of 10 weeks, Thus, there is no evidence that pea mosaic virus is seed transmitted, or that germination is adversely affected.

Factors affecting the transmission by aphids

Pea mosaic virus was transmitted by the aphids *Myzus persicae*, *Macrosiphum pisi*, *M. solanifolii* and *Aphis fabae* but not by *Hyperomyzus staphyleae*.

Table 1 shows the summarized results of seven replications of an experiment to test the effect of preliminary fasting (for 0 and 24 hr.) and varying infection-feeding times (2 min., 1 and 24 hr.) on the transmission of pea mosaic virus by *Myzus persicae*. In each replication five test plants were used per treatment and each plant was colonized with three aphids. The test-feeding time was 24 hr.

TABLE 1. *Effect of preliminary fasting and varying infection-feeding times on the transmission of pea mosaic virus by* Myzus persicae

Preliminary fasting times	Infection-feeding times			Total
	2 min.	1 hr.	24 hr.	
0	18(*a*)	26	22	66
24 hr	30	17	22	69
Total	48	43	44	135

Standard error (*a*) ± 5·2.

The fasted aphids caused most infections after a 2 min. infection feed and their efficiency was reduced when this period was prolonged to 1 or 24 hr. There was no significant difference between the numbers of infections obtained after infection feeds of 1 and 24 hr.; thus the efficiency of the aphids does not progressively decrease with increase in infection-feeding time. Without preliminary fasting, fewer infections were obtained with the 2 min. infection feed than with 1 and 24 hr. This experiment demonstrated that 2 min. infection-feeding and 24 hr. test-feeding periods are sufficient for *M. persicae* to transmit pea mosaic virus, and that preliminary fasting influences the efficiency of the vector provided the infection-feeding time is short; when this time is longer than 1 hr., vectors behave similarly whether previously fasted or not.

A second experiment was done to find how variations in the preliminary fasting periods from 15 min. to 24 hr. affected the transmission of pea mosaic virus by *M. persicae*. Ten test plants were used per treatment and infection-feeding and test-feeding times were 5 min. and 24 hr. respectively. Of ten test plants colonized with aphids that received preliminary fasting periods of 15 min., 1, 4, 16 and 24 hr. respectively, 9, 10, 10, 9 and 9 became infected, suggesting that the full effect of preliminary fasting on vector efficiency is developed within 15 min. These results differ from those obtained with Hyoscyamus 3, potato *Y*, and cucumber 1 viruses (Watson & Roberts, 1939) transmitted by *M. persicae*, for with these viruses the vector efficiency increased in the ratio 1:2:3:5 for preliminary fasting times of 0, 15 min., 1 and 4 hr. It agrees with the behaviour of these viruses when transmitted by *Macrosiphum gei*, and with that described for beet mosaic virus with *Myzus persicae* by Sylvester (1949).

In the transmission of Hyoscyamus virus 3 by previously fasted *M. persicae* the number of infections obtained after 2 min. infection feeding was more than five times that obtained after 1 hr. (Watson, 1938). Similar results were obtained in the transmission of tobacco etch (Kassanis, 1941). With beet mosaic virus (Watson, 1946) infectivity was increased by a factor of 3 or 4. In the first experiment with pea mosaic virus described in this paper, however, although the difference between the numbers of infections obtained by using fasted *M. persicae* after 2 min. and 1 hr. infection feeds was significant, it was smaller than with other viruses cited. Possible reasons for this might be either that the critical period of infection feeding lies

between 2 min. and 1 hr. or that too many aphids were used per plant to show critical differences. In the third experiment, therefore, two additional infection-feeding periods were included between 2 min. and 1 hr. and only two aphids instead of three were used per plant; the preliminary fasting times also were varied. Five test plants were used per treatment. The test-feeding time was 24 hr. with preliminary fasting times of 0, 15 min. and 5 hr., and infection-feeding times of 2, 15, 30 min., 1 and 18 hr. The experiment was replicated four times. The results are given in Table 2.

TABLE 2. *Effect of varying preliminary fasting and infection-feeding times on the transmission of pea mosaic virus by* Myzus persicae

Preliminary fasting times	Infection-feeding times					Total
	2 min.	15 min.	30 min.	1 hr.	18 hr.	
0	2(*a*)	8	6	12	9	37(*b*)
15 min.	16	16	12	11	9	64
5 hr.	14	12	13	10	8	57
Total	32	36	31	33	26	158

Standard errors: (*a*) ± 2·9; (*b*) ± 6·5

The effect of varying preliminary fasting and infection-feeding times on the efficiency of the vectors was the same as in Exps. 1 and 2, there being no difference between the numbers of infections caused by aphids which had fasted for 15 min. and 5 hr. When the feeding time on the infected plants was shorter than 1 hr., the fasting and feeding aphids behaved differently, but when it was longer than 1 hr. both groups were equally efficient. The use of two instead of three aphids per plant did not alter the proportions of infections for 2 min. and 1 hr. infection-feeding periods but apparently increased the effect of preliminary fasting. No critical infection-feeding period was demonstrated by inserting two additional short infection-feeding times—15 and 30 min.—in the experiment.

The relative efficiencies of three species of aphid, *M. persicae*, *Macrosiphum pisi* and *Aphis fabae*, in transmitting pea mosaic virus to two common hosts—pea and broad bean—were compared, keeping the preliminary fasting and test-feeding times

TABLE 3. *Comparison of* Myzus persicae, Macrosiphum pisi *and* Aphis fabae *as vectors, and pea and broad bean as host plants of pea mosaic virus*

Aphids	Pea (infection-feeding times)				Broad bean (infection-feeding times)				Pea + broad bean (infection-feeding times)			
	2 min.	1 hr.	24 hr.	Total	2 min.	1 hr.	24 hr.	Total	2 min.	1 hr.	24 hr.	Total
Myzus persicae	16(*a*)	13	11	40(*b*)	19	11	11	41	35(*c*)	24	22	81(*d*)
Macrosiphum pisi	14	2	4	20	14	4	1	19	28	6	5	39
Aphis fabae	19	8	3	30	18	8	3	29	37	16	6	59
Total	49	23	18	90	51	23	15	89	100	46	33	179

Standard errors: (*a*) ± 1·9; (*b*) ± 3·4; (*c*) ± 2·7; (*d*) ± 4·8.

constant at 5 and 24 hr. respectively. Five test plants were used per treatment with three aphids per plant. The feeding times on the infected plants were 2 min., 1 hr. and 24 hr. respectively. The experiment was repeated four times. The results are summarized in Table 3.

Pea and broad bean were equally readily infected by all three species of aphids. The three species of aphids, however, differed significantly in efficiency as vectors, *Myzus persicae* causing the most and *Macrosiphum pisi* the least infections. These aphids differed, not only in the total number of infections they caused, but also in the rates at which their efficiency decreased with increase of infection-feeding periods. All the three species responded to preliminary fasting and short infection feeding, but the deleterious effects of a prolonged infection feeding were more pronounced with the less efficient vectors than with *Myzus persicae*.

The reason for the three species behaving differently is not known, but it may be relevant that pea and broad bean are usual hosts for *Macrosiphum pisi* and *Aphis fabae* but not for *Myzus persicae*. If aphids feed more continuously, or secrete more digestive enzymes for some other reason, when feeding on a favoured host plant than when feeding on a plant less acceptable, the results are interpretable on the theory that aphids produced virus-inhibitory substances with the enzymes (Watson & Roberts, 1939). More of such substances would be produced by *Macrosiphum pisi* and *Aphis fabae* feeding on broad bean for 1 or 24 hr. than by *Myzus persicae*, and the vector efficiency of the first two would be reduced by long infection feeding more than that of *M. persicae*. No such difference between species would be expected with short infection-feeding times, and all three are equally good vectors after short infection feeding. This hypothesis could account for the slight effect on vector efficiency with *M. persicae* produced by increasing infection-feeding time. The variation in vector efficiency with varying infection-feeding times shown by *Aphis fabae* and *Macrosiphum pisi* here is similar to that shown by *Myzus persicae* in the transmission of other viruses (Kassanis, 1941; Watson, 1938, 1946) in host plants which were acceptable to this aphid.

An experiment was made to test: (1) the effect of varying lengths of the test-feeding times; (2) the persistence of infectivity when aphids are fed continuously after a single infection feed; and (3) whether the length of infection-feeding time has any influence on (1) and (2).

Five test plants were used per treatment with two *Myzus persicae* per plant. The preliminary fasting time was 18 hr. and infection-feeding times were 2 min. and 24 hr. The experiment was repeated five times. For the test feeding, each lot of two aphids was placed first on one plant (*A*) for periods of 5, 15 min., 1 or 2 hr. and then removed to a second plant (*B*) where they were allowed to feed for 24 hr.

Table 4 shows that *M. persicae* can pick up pea mosaic virus from an infected plant in 2 min. and cause infection in a 5 min. test feeding period. The number of infections obtained in the second test plants decreases with increasing length of time spent on the first test plant. Few aphids remain infective for longer than 2 hr. when

TABLE 4. *Effect of varying infection-feeding and test-feeding times on the transmission of pea mosaic virus by* Myzus persicae

Infection-feeding times	Test-feeding times: *A* (5 min.)	*B* (24 hr.)	*C*	*D*	*A* (25 min.)	*B* (24 hr.)	*C*	*D*	*A* (1 hr.)	*B* (24 hr.)	*C*	*D*	*A* (2 hr.)	*B* (24 hr.)	*C*	*D*	Total *A*	*B*	*C*	*D*
2 min.	19	17	36	13	16	8	24	5	19	1	20	1	23	0	23	0	77	26	103	19
24 hr.	6	8	14	3	10	5	15	3	20	2	22	2	20	3	23	3	56	18	74	11
Total	25	25	50	16	26	13	39	8	39	3	42	3	43	3	46	3	133	44	177	30

A = first plant of the series; *B* = second plant of the series; *C* = total number of plants infected in *A* and *B*; *D* = number of plants infected by the same lot of aphids both in *A* and *B*.

allowed to feed continuously and most are non-infective after 1 hr. These results resemble those obtained by Osborn (1937) in experiments on the transmission of pea virus 2 by various aphids.

The numbers of infections caused by aphids given short and long infection feeds after preliminary fasting differ more when the test-feeding time is short than when long. Aphids given short infection feeds appear to become non-infective more rapidly than those given long infection feeds.

In a further experiment the aphids were made to fast both before and after infection-feeding periods of 5 min. and 16 hr. Three *M. persicae* were used per test plant of which there were four per treatment. The test-feeding time was 24 hr. and the post-infection-fasting times were 0, 15 min., 1 and 3 hr. The experiment was repeated three times.

TABLE 5. *Effect of post-infection fasting after varying infection-feeding periods in the transmission of pea mosaic virus by* Myzus persicae

Infection-feeding times	Post-infection fasting times: 0	%	15 min.	%	1 hr.	%	3 hr.	%	Total
5 min.	8	66	7	58	3	25	3	25	21
16 hr.	3	25	5	41	6	50	0	0	14
Total	11		12		9		3		35

Table 5 shows that *M. persicae*, given both preliminary fasting and post-infection fasting, retain the virus for three or more hours if the infection-feeding time is short (5 min.) but not when it is long (16 hr.). The previous experiment showed that, even when *M. persicae* had undergone a preliminary fasting followed by short infection feed, they usually lost their infectivity in 1 hr. of continuous test feeding. Thus both these experiments show that infective aphids lose their infectivity more quickly when feeding than when fasting. Watson (1938) and Kassanis (1941) reported similar results with *M. persicae* for Hyoscyamus virus 3 and tobacco etch viruses respectively.

Table 5 shows that aphids made to fast for a short period after a long infection feed, infected more plants than if they were transferred directly from the infected plants to the test plants. It may be that, during a short post-infection fasting after a long infection feed, the amount of inhibitory substance in the aphid decreases with a consequent increase in vector efficiency.

Effect of consecutive feedings on a series of test plants after a single infection feed

In an experiment made to test the effect of consecutive feeding of aphids after single infection feedings, ten *M. persicae* were given 5 hr. preliminary fasting and 5 min. infection feeding, and then transferred singly to a series of eight consecutive test plants. They were allowed 5 min. feeding on each of the first seven plants and 24 hr. on the last plant. Only three out of the ten aphids caused infection, and these infected the first plant only. These individuals, therefore, lost their capacity to cause infection during the first 5 min. of feeding, but their failure may have been partly due to an unusually low level of infectivity, for in Table 4, groups of two aphids gave thirteen consecutive infections when 5 min. was spent on the first plant, and five when they fed for 25 min. on the first plant. Some of these second infections might have been caused by individuals from a pair which did not feed on the first plants, but it seems unlikely that a large proportion could have done so, and still maintain such a high level of transmission (seventy-seven infections in eighty plants) to the first plants. Had so many aphids failed to feed on the first plants, there would have been more occasions in which both failed to feed on one plant, than is indicated by the results.

Another experiment was made, in which the aphids were given the same preliminary fasting and infection-feeding treatments but only two test plants were used in each series. On the first of these the aphids fed for 3 min. and on the second for 24 hr. These aphids caused seven infections in the first plants and nine in the second plants. The seven which infected the first plants also infected the second. The group obviously had a much higher level of infectivity than those used in the previous experiment, and their ability to infect more than one plant after a single infection feeding was clearly demonstrated.

In further experiments the treatments were again similar to those already described, except that three test plants were used in each series and the test-feeding times were 2, 3 min., and 24 hr. on the first, second and third successive plants of each series. Five of ten aphids tested became infective; one infected all the three plants; one infected the first and third; two infected the second and third, and one infected only the third plant.

The distribution of infected plants in this experiment provides evidence against the hypothesis that the virus is carried mechanically as an external contaminant on the mouthparts of the aphid. If transmission occurred in this manner, the chances of infection would not be expected to increase with increasing length of test-feeding time, and plants in the beginning of a series would not be expected to escape

infection. Further evidence against the hypothesis of external mechanical transmission of pea mosaic virus by aphids is the varying efficiencies of different vector species and the failure of *Hyperomyzus staphyleae* to transmit it.

In its general behaviour pea mosaic virus resembles the 'non-persistent' viruses as defined by Watson & Roberts (1939). The differences are that the vectors react more rapidly to preliminary fasting with pea mosaic, and that vector efficiency both fails to increase with increase of the preliminary fasting period and remains constant for infection-feeding periods of more than 1 hr.

Pea enation mosaic virus

Symptoms and host range

The first symptom of pea enation mosaic appears about 10 days after infection, taking a few days longer in winter than in summer. The first symptom in pea is usually a slight vein-clearing in newly developing leaves and stipules, followed in a few days by the development of enations on the veins of their lower surfaces, but sometimes there is a yellow spotting instead of vein-clearing, or the first symptoms are the enations.

Pea enation mosaic is more injurious than pea mosaic. Infected plants are stunted; the terminal bud sometimes ceases to grow and lateral shoots are produced. The pods are reduced in size and become rough and wrinkled. Infected soy-bean plants first show vein-clearing, which is followed by a mild mosaic but no enations are produced. Leaves of infected sweet pea are rough and uneven, with light yellow transparent streaks, and produce enations on the undersurface. In crimson clover, vein-clearing is followed by yellow streaking or mottling and later by well-developed enations along the veins on the undersurfaces of leaves. Several varieties of broad bean, red clover, spring vetch, tobacco (var. White Burley), *Nicotiana glutinosa*, French bean, vars. Prince and Bountiful, white clover and subterranean clover were found to be insusceptible.

The properties of the virus *in vitro* were not studied because of the great difficulties in getting transmission by inoculation with sap. When pea plants were rubbed with undiluted sap from infected plants only occasional plants became infected, even when carborundum was incorporated in the inoculum.

Transmission by aphids

The aphids *Macrosiphum pisi*, *Myzus persicae* and *Macrosiphum solanifolii* transmitted pea enation mosaic virus, the first being the most efficient vector and the third the least, and *Hyperomyzus staphyleae* did not. The first experiments with *Macrosiphum pisi* suggested that it was less readily transmitted than pea mosaic virus and that the efficiency of the vectors was not increased by preliminary fasting. Of 160 seedlings colonized with single aphids that had received various combinations of preliminary fasting and different infection-feeding times, only ten became infected,

and these were colonized with aphids that received infection-feeding times of either 6 or 24 hr. This suggested that pea enation mosaic was a persistent virus (Watson & Roberts, 1939) and that there might be a period after the vector fed on an infected plant before it could infect a healthy one.

Smith & Bonequet (1915) first reported an 'incubation' period with the transmission of sugar beet curly-top virus by *Eutettix tenellus* Baker. Since then a 'latent' period in the vector has been demonstrated with several other plant viruses. These are mostly transmitted by leaf-hoppers or thrips, but Elze (1927) and Smith (1931) found evidence of a 'latent' period in the transmission by *Myzus persicae* of potato leaf-roll virus, and Osborn (1935, 1938*a*, *b*) stated that pea virus 1 required a 'latent' period in the aphids *Macrosiphum pisi* and *M. solanifolii*. Experiments were made, therefore, to see whether there is a 'latent' period and how its duration is affected by varying feeding conditions. Aphids were allowed to feed on infected plants for varying periods and then transferred to successions of test plants on which they remained for different periods. Garden peas (var. Lincoln) were used throughout as test plants and sources of infection. Temperature may influence the length of the 'latent' period of viruses in their vectors (Kunkel, 1937; Osborn, 1935), and in all experiments the temperature in the insectary was kept at about 65° F.

Five colonies each of twenty-five *M. pisi*, were given 16 hr. infection feeding and then transferred to seven successive plants. The test-feeding time on each plant varied from 2 to 24 hr.

TABLE 6. *Infections obtained with* Macrosiphum pisi *fed on plants infected with pea enation mosaic virus for* 16 *hr. and then transferred to a succession of healthy plants*

Successive test feedings	Lengths of successive test feedings (hr.)	Total times since end of infection feeding (hr.)	Succession of test plants Colonies of aphids 1	2	3	4	5
I	2	2	−	−	−	−	−
II	3	5	−	−	−	−	−
III	4	9	−	−	−	−	−
IV	14	23	−	−	−	−	−
V	9	32	−	−	−	−	−
VI	16	48	+	−	−	−	+
VII	24	72	+	−	+	−	+

+ = plant infected; − = plant not infected

Table 6 shows that only three out of five colonies of aphids caused infections, and the first five plants of the series all remained healthy. The first and fifth colonies caused infections after a period of between 32–48 hr. of leaving the infected plant and the third colony after one of 48–72 hr. The experiment demonstrated clearly that *M. pisi*, given an infection-feeding time of 16 hr., could not transmit pea enation mosaic virus immediately, but required a 'latent' period whose duration varied in different aphids.

In a similar experiment using *Myzus persicae* as the vector, five colonies of twenty-five aphids were given infection feeding of 18 hr., and then transferred to series of five consecutive plants. They fed for 2 hr. on the first two plants, 4 hr. on the third, sixteen on the fourth, and twenty-four on the fifth. No infections occurred on the first four plants in this series, but four out of the last five plants to be colonized were infected. With *M. persicae*, as with *Macrosiphum pisi*, no infections occurred until 24 hr. after the aphids left the infected plants.

An experiment was made to test the effect of shorter infection-feeding time on the length of the latent period and to determine how long an aphid would retain the virus after such an infection feed. Five colonies of *M. pisi* and five of *Myzus persicae* were used. Each colony consisted of twenty aphids.

TABLE 7. *Infections obtained with* Macrosiphum pisi *and* Myzus persicae *fed on plants infected with pea enation mosaic for 2 hr. and then transferred to a series of healthy plants*

Successive test feedings	Length of successive test feedings (hr.)	Total times since end of infection feed (hr.)	Succession of test plants Colonies of aphids: *M. pisi* 1	2	3	4	5	*M. persicae* 1	2	3	4	5
I	4	4	−	−	−	−	−	−	−	−	−	−
II	20	24	+	−	−	−	−	−	−	−	−	−
III	4	28	+	−	−	−	−	−	−	−	−	−
IV	46	74	+	+	−	−	−	−	−	−	+	−
V	66	140	+	−	−	−	−	−	−	+	+	−
VI	48	188	+	−	+	−	−	−	−	+	+	−

+ = plant infected; − = plant not infected.

The results (Table 7) show that an infection feed of 2 hr. is sufficient for an aphid to acquire the virus. The 'latent' period after this infection feed in one colony of *Macrosiphum pisi* was between 4 and 28 hr. and this colony caused infections in all the remaining four plants of its series. The minimum 'latent' periods with two other colonies of *M. pisi* were 28 hr. while another caused no infection before 140 hr. Both species of aphid retained the virus after a single 2 hr. infection feed for at least 140 hr. This experiment also demonstrated that the virus could be transmitted during a test-feeding period of only 4 hr. once the aphids had become infective.

The significance of the 'latent' period of a virus in insect vectors is not yet understood, although various hypotheses have been put forward by different investigators. These are: (1) that the virus undergoes some sort of developmental change inside the vector before it can be transmitted; (2) that when the vector feeds on an infected plant it obtains insufficient virus to be immediately infective and that the 'latent' period is the time during which the initial virus multiplies sufficiently to make the vector infective; (3) that there is a minimum time needed for the virus to pass through the wall of the alimentary canal, and travel along the blood stream to

the salivary glands of the vector before it can be transmitted. Bawden (1943) has pointed out that on all these hypotheses the length of the 'latent' period would be expected to vary with the length of infection-feeding time. The longer an insect feeds on the source of infection, the shorter should be the 'latent' period. When the time given for the infection feeding is longer than the 'latent' period necessary with a short infection feed, the vector should be able to cause infection immediately it leaves the diseased plant. A series of experiments was made, therefore, to test whether or not a long infection-feeding period would, in fact, affect the length of the 'latent' period.

Five colonies of *M. pisi* each consisting of fifteen aphids were fed on the infective source for 3 days. Each colony was then transferred to five successive healthy plants. The time spent by each colony of aphids on each successive healthy plant is shown in Table 8.

TABLE 8. *Infections obtained with* Macrosiphum pisi *fed on plants infected with pea enation mosaic virus for* 3 *days and then transferred to a succession of healthy plants*

Successive test feedings	Lengths of successive test feedings (hr.)	Total times since end of infection feeding (hr.)	Succession of test plants Colonies of aphids				
			1	2	3	4	5
I	2	2	−	−	−	−	−
II	4	6	−	−	−	−	−
III	16	22	−	+	−	+	−
IV	4	26	−	+	−	+	+
V	24	50	−	+	+	+	+

+ = plant infected; − = plant not infected.

The results show that, even after an infection feed lasting for 3 days, the aphids did not infect the plants on which they fed immediately they had finished their infection feeding. Two of the colonies caused infections after a 'latent' period of between 6 and 22 hr.; with two other colonies, the minimum lengths of this period were 22 and 26 hr.

Five colonies of *M. pisi*, each consisting of seven aphids, were fed on the infected plants for 68 hr. before they were transferred to the successive test plants. Only three of the colonies caused any infections and none did so in less than 24 hr.

Ten colonies, each consisting of fifteen individuals of *M. pisi*, were divided, half being allowed to feed on the infected plant for 48 hr. and the other half for 2 hr. Each colony was then transferred successively to a series of seven test plants. Among the group receiving an infection feed of 48 hr., some aphids were noticed to stop feeding before the end of the 48 hr. period and these were separated into one colony (colony 5).

Table 9 shows that the two groups of aphids treated similarly, except for the variation in infection-feeding times, both failed to cause infection until 20 hr. after leaving the

TABLE 9. *Infection obtained with* Macrosiphum pisi *fed for varying periods on plants infected with pea enation mosaic and then transferred to a succession of healthy plants*

Successive test feedings	Lengths of successive test feedings (hr.)	Total times since end of infection feeding (hr.)	Succession of healthy plants: Infection feed 2 hr. Colonies of aphids 1	2	3	4	5	Infection feed 48 hr. Colonies of aphids 1	2	3	4	5
I	6	6	−	−	−	−	−	−	−	−	−	−
II	14	20	−	−	−	−	−	−	−	−	−	+
III	8	28	−	+	−	−	−	−	+	−	−	+
IV	20	48	−	+	+	−	−	+	+	+	+	+
V	22	70	−	+	+	+	+	+	+	+	+	+
VI	48	118	+	+	+	+	+	+(5)	+(7)	+(7)	+(5)	+(9)
VII	48	166	+	+	+	+	+	+(4)	+(6)	+(6)	+(1)	+(9)

+ = plant infected; − = plant not infected. The numbers in parentheses indicate numbers of aphids in the colony on the particular plant.

infected plant. Colony 5 from the 48 hr. infection-feeding group, consisting of aphids that had wandered from the infected plant before the end of the 48 hr. infection-feeding period, and therefore whose actual length of infection-feeding time was uncertain, caused infection within 6–20 hr. test feeding. In different colonies in the 2 hr. infection-feeding group, the apparent 'latent' period varied between a minimum exceeding 20 and a maximum of less than 118 hr.; except for colony 5, in the 48 hr. infection-feeding group it was in one colony between 20 and 28 hr. and in the other three between 28 and 48 hr.

These results suggest that the 'latent' period is not abolished by prolonging the infection-feeding time, and there is little evidence that it is appreciably shortened. Whether aphids had fed for 2 or 48 hr. on the infected plants, they still caused no infections in the first few hours on healthy plants. Thus it would seem that the latent period begins when the aphids cease feeding on diseased plants rather than when they start. There is, however, no fixed 'latent' period and the time taken for different individuals to become infective varies considerably. There is some evidence that those with the shortest latent periods also cause most infections, but more work will be needed to demonstrate this conclusively. No interpretation can be given for the 'latent' period, but as Bawden (1943) has suggested, it may be the time necessary for vectors to free the virus from materials which are imbibed from infected plants and interfere with its transmissibility.

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THE SPREAD OF VIRUS DISEASES TO SINGLE POTATO PLANTS BY WINGED APHIDS

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Young potato plants in pots exposed in the open near plots of potatoes for limited periods at intervals during the summer, became infested with large numbers of winged aphids only during warm, calm and dry weather. Although visited by aphids during May and June, when much of the spread of viruses occurred in nearby potato crops, few of the potted plants became infected. Most potted plants became infected in July when alate aphids were leaving neighbouring potato crops. Widely different proportions of the exposed plants became infected in different years; in two of the three years, many more plants were infected with virus *Y* than with leaf roll virus.

In the better potato-seed producing areas of Britain what spread there is of potato virus *Y* and leaf roll virus seems to occur late in the season, and the health of potato stocks can be maintained by the early removal of any infected plants. Doncaster & Gregory (1948) found that in parts of south-east England, both viruses spread early in the season and that healthy plants usually contracted viruses from infected plants within the same crop and not from distant sources. They concluded that the winged migrants which started the summer infestation of aphids in potato crops caused many more infections in proportion to their numbers than did either the large apterous populations which developed later in the season or the numerous alatae usually produced in July or August.

To obtain more information on the time of year during which alate aphids spread viruses, batches of young healthy potato plants in pots were exposed in the open for periods of 2 or 3 weeks at intervals during the summer. They were examined for aphids and after exposure were fumigated and grown to maturity in a glasshouse. Their progeny was examined for symptoms of leaf roll and rugose mosaic during the following season.

1944 Experiment

Batches of Stock Seed potatoes (variety King Edward) were planted in 7 in. porous pots at intervals during the summer, and when the plants were about a fortnight old they were reduced to a single stem and were put on bare ground in an area of allotments about 3 yards from experimental plots of potatoes containing plants infected with leaf roll virus and potato virus *Y*.

Half of each tuber was planted as a control to ensure that no tuber with virus disease was included in the experiment.

Of the 263 plants put out during the experiment, 20·5 % became infected with potato virus *Y* and 1·9 % with leaf roll. The main spread of virus *Y* was to batches

TABLE 1. *Aphids recorded on exposed potted potato plants and percentage infection with virus disease, 1944–6*

Batch	Exposure: No. of plants	Exposure: Days	Date	*M. persicae* Al.	*M. persicae* Ap.	*M. persicae* Ny.	*A. solani* Al.	*A. solani* Ap.	*A. solani* Ny.	*M. euphorbiae* Al.	*M. euphorbiae* Ap.	*M. euphorbiae* Ny.	*A. rhamni* Al.	*A. rhamni* Ap.	*A. rhamni* Ny.	Virus (%) *Y*	Virus (%) Leaf-roll
			1944														
1	50	17	23 May	12	0	2	1	0	1	2	0	0	0	0	0		
		(17 May–	26 May	11	0	28	1	0	0	4	0	10	0	0	0		
		2 June)	30 May	20	8	120	0	0	5	20	5	100	0	0	0	2	2
			1 June	22	24	236	0	0	4	22	18	167	0	0	0		
			2 June	19	13	90	0	1	1	19	11	131	0	0	0		
2	50	19	7 June	2	0	2	1	0	0	2	0	0	0	0	0		
		(3 June–	12 June	2	0	13	2	0	3	1	1	6	3	0	8		
		21 June)	16 June	12	0	27	3	0	21	3	2	20	5	0	21	4	0
			21 June	20	2	125	5	0	25	8	3	84	4	1	50		
3	47	21	24 June	15			0			3			0				
		(22 June–	30 June	8	3	116	1	0	4	3	6	23	2	0	6		
		12 July)	5 July	24	2	229	2	0	5	5	5	63	2	0	14	44	0
			7 July	18	32	304	1	1	3	26	12	78	1	1	13		
			11 July	15	42	692	1	0	10	12	12	164	2	5	45		
4	50	22	14 July	4	4	3	0	0	0	2	1	3	0	0	0		
		(13 July–	19 July	48	5	213	0	0	1	39	4	141	2	0	14		
		3 Aug.)	21 July	35	2	277	1	0	3	24	2	181	1	0	18	63	6
			25 July	15	12	385	0	0	1	8	3	128	1	0	28		
			1 Aug.	2	4	49	0	0	0	1	3	26	0	1	14		
5	50	41	31 Aug.	0	0	0	0	0	0	0	0	0	0	0	0	2	2
		(4 Aug.–															
		12 Sept.)															
6	16	23	16 Sept.	0	0	0	0	0	0	1	0	2	0	0	0		
		(13 Sept.–	25 Sept.	0	0	0	0	0	0	0	0	0	0	0	0		
		5 Oct.)	28 Sept.	0	1	3	0	0	0	0	0	0	0	0	0	0	0
			3 Oct.	0	0	6	0	0	0	0	0	0	0	0	0		
			1945														
1	50	15	18 May	0	0	0	0	0	0	0	0	0	0	0	0		
		(16–30	22 May	0	0	1	3	0	9	0	0	2	0	0	0		
		May)	25 May	0	0	2	0	0	8	0	0	1	0	0	6	2	0
			28 May	1	2	9	0	0	7	0	0	3	0	0	6		
2	50	14	4 June	0	0	0	0	0	0	0	0	0	0	0	0		
		(1–14	7 June	0	0	0	0	0	0	0	0	3	0	0	0		
		June)	11 June	0	1	2	0	0	1	0	0	0	0	0	0	0	2
3	50	18	18 June	0	4	9	1	1	2	0	0	0	0	0	0		
		(15 June–	21 June	10	6	100	0	1	7	0	0	5	0	0	0		
		2 July)	25 June	11	15	349	1	4	7	3	1	36	0	0	0	4	10
			28 June	11	9	507	0	4	14	4	1	13	0	0	0		
4	50	14	5 July	11	4	36	0	0	1	1	0	5	0	0	0		
		(3–16	10 July	53	12	451	6	3	35	10	2	53	1	0	24	24	18
		July)	13 July	64	23	676	5	2	25	25	3	139	0	0	16		
			1946														
1	47	16	10 June	0	0	0	0	0	0	0	0	0	0	0	0		
		(9–24	14 June	5	0	1	0	0	0	0	0	0	0	0	0		
		June)	18 June	1	1	7	0	0	0	0	0	0	0	0	0	2	0
			20 June	4	3	22	0	0	0	0	1	0	0	0	0		
			24 June	5	1	82	0	0	0	0	1	2	0	0	0		
2	50	17	29 June	1	0	0	0	0	0	0	0	1	1	0	0		
		(25 June–	7 July	0	0	3	0	0	0	0	1	4	1	0	6	4	0
		11 July)	11 July	1	1	10	0	0	0	1	3	17	0	2	34		
3	50	16	15 July	0	0	0	0	0	0	2	1	1	0	0	0		
		(12–27	19 July	0	0	1	0	0	0	1	1	11	0	0	0	6	0
		July)	23 July	0	0	1	0	0	0	2	0	24	0	0	0		
			27 July	1	0	2	0	0	0	2	0	37	0	0	0		
4	50	16	(28 July–12 Aug. 8 examinations, no aphids seen													0	0
5	49	16	19 Aug.	2	0	1	0	0	0	0	0	0	1	0	0		
		(14–29	27 Aug.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Aug.)															
6	21	15	3 Sept.	0	0	0	0	0	0	0	0	0	0	0	0		
		(29 Aug.–	7 Sept.	0	0	2	0	0	0	0	0	0	0	0	0	0	0
		12 Sept.)	13 Sept.	0	0	0	0	0	0	0	0	0	0	0	0		

3 and 4, between 22 June and 3 August (Table 1). The mean time for plants of batch 3 to show symptoms of leaf-drop streak after being returned to the glasshouse was 38 days, compared with 21 days for batch 4. As these batches were exposed for 21 and 22 days respectively and temperatures during these periods were similar (Table 4), it can be inferred that most plants became infected during the last week of exposure of batch 3 and the first of batch 4, that is during the first 3 weeks of July. This was the period when most alate aphids were present on the plants (the aphids were counted every day) and it coincided with the decline of the aphid infestation on neighbouring potato crops (Table 2) and with the largest catches on an adjacent insect trap (Table 3). Tables 2 and 3 are compiled partly from data given by Doncaster & Gregory (1948).

Although many alate *Myzus persicae* (Sulzer) and *Macrosiphum euphorbiae* (Thomas) visited all the first four batches, it was only during the time that aphids were leaving potato crops in large numbers that many of the plants became infected. There were

TABLE 2. *Counts of aphids per* 100 *leaves on the potato crops, Rothamsted Farm,* 1944–6

	June				July				August				Sept.		
Week ...	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3
1944															
M. persicae	33	—	139	—	1708	—	253	2	—	0	—	0	—	0	—
A. solani	0	—	2	—	1	—	0	0	—	0	—	0	—	0	—
M. euphorbiae	3	—	15	—	291	—	58	0	—	0	—	0	—	0	—
A. rhamni	1	—	12	—	30	—	18	1	—	0	—	0	—	0	—
1945															
M. persicae	7	—	3	—	106	—	95	5	—	10	—	18	—	—	—
A. solani	0	—	0	—	0	—	0	0	—	0	—	0	—	—	—
M. euphorbiae	0	—	1	—	12	—	21	0	—	1	—	2	—	—	—
A. rhamni	0	—	1	—	23	—	0	2	—	0	—	0	—	—	—
1946															
M. persicae	11	10	—	34	—	77	—	13	—	14	—	16	—	—	0
A. solani	4	0	—	0	—	1	—	0	—	0	—	0	—	—	0
M. euphorbiae	4	0	—	0	—	16	—	4	—	3	—	1	—	—	0
A. rhamni	5	1	—	15	—	13	—	5	—	4	—	13	—	—	0

TABLE 3. *Trap catches of aphids near the pot experiments,* 1944–5

	May		June				July				August				Sept.				Oct.
Week ...	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1
1944																			
M. persicae	1	1	1	2	8	3	23	19	9	1	0	0	0	0	0	0	0	0	4
A. solani	2	3	0	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0
M. euphorbiae	0	0	0	2	0	1	9	4	1	0	0	0	0	0	0	0	0	0	0
1945																			
M. persicae	2	0	0	0	5	1	17	14	4	2	1	0	0	0	—	—	—	—	—
A. solani	0	0	0	0	0	0	1	3	0	0	1	0	0	0	—	—	—	—	—
M. euphorbiae	0	0	0	0	0	0	3	2	2	0	0	0	0	0	—	—	—	—	—

Table 4. *Mean temperatures, rainfall and number of days with rain during the experiments*

Mean temperatures, ° F.

Batch	1944	1945	1946
1	56·2	53·9	55·3
2	54·9	56·6	61·1
3	59·3	58·5	60·7
4	62·1	62·9	60·3
5	60·6	—	57·0
6	53·4	—	56·6

Rainfall and number of days with rain/no. of days exposed

	1944		1945		1946	
Batch	Rain	Days	Rain	Days	Rain	Days
1	0·35	2/17	1·37	8/15	1·31	12/16
2	0·57	8/19	1·35	10/14	1·14	5/17
3	1·80	12/21	0·85	9/18	1·17	8/16
4	0·44	6/22	2·43	5/14	1·83	9/16
5	4·51	21/41	—	—	1·97	11/16
6	0·85	10/23	—	—	2·63	12/16

as many nearby sources of leaf roll as of virus *Y*, and the difference between the numbers of plants contracting the two viruses is not explicable by variations in numbers of infection foci.

Throughout the experiment the plants were colonized by large numbers of alate aphids only during calm, warm periods. The first part of the fourth period was calm and dry and the numbers of alatae increased rapidly. The period 26–29 July was wet and calm and an attack by parasitic fungi and numerous predators rapidly reduced the colonies. Aphids elsewhere must have been similarly attacked, for few alatae colonized the plants in the fifth and sixth periods, although the weather was often favourable for aphid flight.

Few apterous aphids reached the plants from the potatoes a short distance away or from heavily infested cruciferous weeds growing close to the pots. The apterae counted on the plants mostly developed from nymphs deposited by visiting alatae. Early in the fourth period, however, five adult apterae were counted which could not have developed on the plants and must have moved from neighbouring plants, or they may have dropped from plants when the previous batch was being removed.

1945 Experiment

The first two batches were 10 ft. from a plot of potatoes with leaf-roll virus; the plot was partly sheltered by buildings. The remaining batches were exposed on a more open site. Counts of aphids were made three or four times during each exposure of about a fortnight (Table 1).

Of the 200 plants put out, 7·5 % became infected with virus *Y* and 7·5 % with leaf

roll. The main spread of virus *Y* was to batch 4 and of leaf roll to batches 3 and 4. These were the only batches in 1945 which became heavily infested with aphids; colonization by alatae occurred between 21 June and 13 July, particularly during the second week of July when the infestation by *Myzus persicae* on neighbouring potato crops was declining (Table 2), and when large catches were taken on an insect trap near the pot experiment (Table 3).

The weather during the exposure of batch 1 was calm, though frequently wet (Table 4), and few aphids visited the potatoes. The second period was very windy as well as wet, and again few aphids visited the plants. The first part of the third period was dry and frequently calm. A few adult apterous *M. persicae* and *Aulacorthum solani* (Kalt.) from weeds colonized the plants in batches 3 and 4 soon after they were put out (Table 1). Alate *Myzus persicae* arrived in the middle of the third period but not towards the end, the last 6 days being cool, wet and windy. The first 6 days of exposure of batch 4 were warm, calm and dry and coincided with the main migration from the field potatoes. This batch was heavily colonized by *M. persicae* and *Macrosiphum euphorbiae*. Heavy rain during the night of 14–15 July (1·8 in.) did not reduce the number of aphids on the plants.

1946 Experiment

This was similar to the previous experiments, but the plants were not reduced to a single stem. Fewer aphids occurred on potatoes than in the previous summers and there was no dispersal of large numbers of winged migrants from the neighbouring potato crops (Table 2). Only two *Myzus persicae* and one *Aulacorthum solani* were caught on a trap near the exposed plants during the whole period of the experiment.

Of the 267 plants put out 2·2 % became infected with potato virus *Y*, none with leaf roll. The first period was calm but wet; the first part of the second period was windy, the last 5 days were warm, calm and dry, but there were few aphids flying to colonize the plants. The rest of the season was frequently wet.

Discussion

The experiments confirmed that large migrations by alate aphids take place only during warm, calm and dry periods, but small numbers of alatae flew under less favourable conditions. Most of the aphids on the plants developed from nymphs deposited by visiting alatae, but a few adult apterae were found on some plants soon after exposure. These may have dropped to the ground when the previous batch was moved or they may have crawled from neighbouring plants. Clearly there was little movement by apterae to the potted plants, although they were only a few feet from infested potatoes and weeds.

Within neighbouring potato crops during the years 1944–6, 30–40 % of the total spread of disease occurred before the large apterous population and summer migrants had developed. This early spread was probably done by the spring migrants

to the crops (Doncaster & Gregory, 1948; Broadbent & Gregory, 1948). During this early period only 2–4 % of the potted plants became infected, despite the proximity of diseased stocks. Doncaster & Gregory (1948) showed that the plants nearest to a diseased plant were the most likely to become infected, and that the chances of infection declined rapidly with increasing distance from the infected plant. These facts, and the small proportion of potted plants exposed early in the season that became infected, suggest that the winged spring migrants make short flights within a limited area when once they have found a suitable crop. The frequency of these flights will depend upon the weather and on the distance flown by the aphids before they found the crop. Young alatae fly more frequently than older aphids (Moericke, 1941; Broadbent, 1949), and migrants which soon find hosts are likely to infect more plants than those which have flown a considerable distance.

In England the summer dispersal migrants usually leave the potato crop in July or early August and the decline in population which follows, and the infrequent occasions when they are seen depositing nymphs, suggest that they are seeking a different crop. Nevertheless, these were the aphids which colonized and infected the exposed single potato plants most heavily, so presumably only the maturing potato plants had become unpalatable, causing the production of a large proportion of alatae which leave the crop. Moericke (1941) found that, when a late colonization of an area occurred, the older potato crops were little infested, whereas a late planted crop was almost killed by the large number of aphids which developed on it.

The summer migrants in 1944 and 1945 were much more numerous than the spring migrants, but despite the early-infected plants which probably formed additional sources of infection by July, their activity and that of the apterous populations did not more than treble the amount of disease in the field crops. It is not known if the relatively small spread of disease at this time was the result of a reduced susceptibility in the healthy plants, a lesser concentration of virus in the diseased plants or of a different behaviour pattern exhibited by emigrant aphids from that shown by immigrants. That the virus which spreads in mid-summer is carried by alatae is suggested by (1) this was the time at which most potted plants became infected, (2) in experiments in 1946, only the places with high trap catches of alate *Myzus persicae* in July and August showed a pronounced increase in leaf roll in the second half of the season (Broadbent & Gregory, 1948), and (3) there is a high correlation between number of alate *M. persicae* trapped and the spread of virus diseases, especially of leaf roll (Broadbent, 1950). It would seem that apterae play little part in disseminating potato virus diseases, even within the crop. Czerwinski (1943) found that apterae moved frequently, but rarely moved further than a few plants from their origin. If the source was a diseased plant it is likely that its neighbours would be among those infected already by alatae, hence the apparent ineffectiveness of apterae in spreading virus in the field.

Contrasting the spread of viruses in neighbouring crops and to the potted plants (Table 5), it is seen that in 2 of the 3 years a bigger percentage of potted plants than

field plants contracted virus *Y*, whereas leaf roll virus always spread much more in the field crops.

TABLE 5. *The spread of viruses in farm potato crops and to single plants*

	1944		1945		1946	
	Virus *Y*	Leaf roll virus	Virus *Y*	Leaf roll virus	Virus *Y*	Leaf roll virus
Farm*	7·1	22·2	5·4	18·3	8·3	16·9
Pots†	20·5	1·9	7·5	7·5	2·2	0

* Mean percentage infection within fifteen plants of diseased source (from Doncaster & Gregory (1948) and Broadbent & Gregory (1948)).

† Mean percentage infection in all batches.

The variety Majestic which was grown on the farm is less susceptible to infection with virus *Y* than the variety King Edward used in the pot experiments, but there is no great difference in the susceptibility of the two varieties to leaf roll virus (Bawden & Kassanis, 1946). In 1946 there were very few summer migrants and a small proportion only of the exposed plants became infected. The high percentage of plants which contracted virus *Y* during July in 1944 and 1945 was to be expected, not only because a susceptible potato variety was used, but because the plants were exposed in an isolated strip and would, as van der Plank (1948) has pointed out, be much more likely to become infected than plants in a similar strip in the middle of a field crop. There was, however, no obvious reason why there was so little spread of leaf roll, especially in 1944 when aphids were numerous and the virus spread considerably in the farm crops. Leaf roll virus might be expected to be spread by alatae more than virus *Y* because of the persistence of leaf roll virus in the aphids and consequently their capacity to infect several plants in succession. In 1944, eleven times more of the exposed plants contracted virus *Y* than leaf roll virus, despite the adjacent plot of potatoes with leaf roll, whereas in 1945 the same variety of potato contracted as much leaf roll virus as virus *Y*. These differences might have been caused by changes in susceptibility in the plants as a result of growth in pots, shortage of water or some other factor, although there is no evidence for this. If the plants had been distasteful and the aphids had stayed a short time only, leaf roll virus might not have been transmitted, but the plants became heavily infested with alatae and their progeny in both years. The counts of alate *M. persicae* (Table 1) suggest that colonization occurred in three waves in 1944 and two in 1945. The aphids which infested the plants in 1944 may have come mainly from plants with virus *Y*, whereas in 1945 a much bigger proportion of the infective aphids originated on plants with leaf roll.

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VIRUS DISEASES OF CACAO IN WEST AFRICA

VI. VECTOR INVESTIGATIONS

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(With 1 Text-figure)

Increasing the number of *Pseudococcus njalensis* per test plant increased the infection rate with cacao virus 1A and 1M to 95% with twenty-five insects. Young leaves with the red vein-banding symptoms were better sources of virus than mature leaves, and there was evidence of virus localization in the plant. It was estimated that *Ps. njalensis* nymphs took about 16 min. to penetrate plant tissues with their stylets.

Infection rates increased with time of test feeding up to 50 min. The virus was non-persistent in the feeding insect, but persisted up to 36 hr. in insects starved after the infection feed. Starvation before the infection feed increased the infection rate with infection feeds up to 10 hrs., after which the infection rate with pre-starved insects declined to the level attained by unstarved insects.

Factors affecting virus transmission

Before an intensive study of vector-virus relationships could be started, the causes of variation in the results obtained by Posnette & Strickland (1948) required investigation. Of the suspected causes, the occurrence of biological races of vectors (transmitting and non-transmitting strains) is discussed in the next paper (Posnette, 1950). The effect of using different numbers and instars of vectors, and of using test plants and source plants of different ages and stages of infection, will be described here in so far as they bear on infection rate.

The vector used was *Pseudococcus njalensis* Laing and the viruses were *Theobroma* virus 1A (Posnette, 1947*a*) and *Theobroma* virus 1M, which has been found only near Mampong about 45 miles south of Tafo. Virus 1M causes symptoms distinct from virus 1A, and from plant-protection tests they seem not to be closely related (Todd, unpubl.). Except in experiments on the age and condition of test plants, cacao beans with one cotyledon removed were used for test feeding (Posnette, 1947*b*). The terminology used is that of Watson (Watson, 1936; Watson & Roberts, 1939; Watson, 1946). When insects, which have been feeding on a source of virus, are transferred to a healthy plant, the period of subsequent feeding is called the *test-feeding period*. When insects which have not previously fed on a virus-infected plant do so, the period from the time when they are placed on the source plant to the time of their removal is termed the *infection-feeding period*. The proportion of test plants infected is termed the *infection rate*.

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(1) *The effect of insect number on virus transmission*

For planning experiments it was necessary to determine the number of insects required to infect a given percentage of test plants under 'optimum' conditions. The effect of increasing the number of nymphs of *Pseudococcus njalensis* from one to thirty per test plant was investigated with all other factors at the presumed optimum (on the basis of previous work). The insects were reared on seedlings of the same age (1 month) and infected for the same period. As a precaution against possible variations in the transmitting powers of insects, the gravid females were colonized on the virus source plants at random, so that each colony of nymphs was derived from several mealybug sources. To guard against variations between the source plants used, the insects from any one source plant were equally distributed among the different treatments in any experiment. This procedure was necessary because sufficient insects for one experiment could not be reared on a single source plant.

In the first experiment, the percentage of test plants infected with virus 1 M rose from 18 with one insect to 53 with five and 77 with ten per test plant. As increases of one or two insects did not affect the infection rate greatly and ten insects per bean did not give 100 % infection, the experiment was repeated using increments of five insects. The results are given in Table 1.

Table 1. *The effect of increasing insect number on infection rate of* Theobroma *virus* 1 M

Exp. no. ...	1	2	3	4	5	6		
Insect number per test plant	No. of infections out of ten						Total	Mean
1	1	3	1	0	1	0	6	1·0
5	4	7	3	7	5	3	29	4·83
10	9	7	8	7	2	3	36	6·0
15	7	8	10	7	7	3	42	7·0
20	9	9	7	9	9	5	48	8·0
25	9	10	10	10	10	7	56	9·33
30	9	10	10	10	9	7	55	9·15

The difference between totals required for significance at the 0·1 % point is 17·3; at the 5 % point 9·7. The difference between Exp. 6 and the other five is significant at the 0·1 % point, suggesting that either the insects used were less efficient than those of the other lines or the source plants contained less available virus.

A similar experiment with *Ps. njalensis* and *Theobroma* virus 1 A is recorded in Table 2. The difference between totals required for significance at the 5 % point is 7·94.

With both viruses a 95 % infection rate was obtained using twenty-five insects per test plant, but the infection rates with the two viruses differed when few insects were used. As the experiments were done by the same person with randomized samples of the insect population and source plants of the same age under the same

conditions, this difference in infectivity probably reflects a difference between the viruses either in invasiveness, availability or concentration in the source plants.

TABLE 2. *The effect of increasing insect number on infection rate of* Theobroma *virus* 1 A

Exp. no. ...	1	2	3	4	5	6		
Insect number per test plant	No. of infections out of ten						Total	Mean
1	1	3	1	0	1	0	6	1·0
5	7	9	5	3	3	5	32	5·3
10	9	10	6	5	5	7	42	7·0
15	9	10	6	6	9	8	48	8·0
20	10	9	9	8	10	8	54	9·0
25	10	10	9	9	10	9	57	9·5
30	10	10	9	10	10	8	57	9·5

The 'mass-action' effect

The possibility of insects injecting sub-minimal infecting doses of viruses which accumulate, so that groups of insects might infect a plant although none could alone, has often been postulated (Carsner & Lackey, 1929). Watson (1936), and Watson & Roberts (1939) have shown that their data for the effect of increasing insect numbers on transmission of *Hyoscyamus* virus III and sugar-beet mosaic virus obeyed the laws of probability and that no increase owing to 'mass-action' effect was demonstrable. Storey (1938) obtained a similar result with *Cicadulina mbila* and maize streak virus, whereas Giddings (1946) has presented evidence of a 'mass-action' effect in the transmission of sugar-beet curly-top virus by *Eutettix tenellus*. His evidence related to the effect of single 'doses' of virus at different strengths, and he concluded that the effect of plant resistance was overcome by large 'doses' of virus. His conception of the 'mass-action' effect in viruses differed from that of Watson and Story in that he considered the introduction of virus into one feeding place on the plant by one insect, whereas they considered the effect of several submininal 'doses' introduced at various points on the plant. Sylvester (1949) showed that the number of feeding punctures made by each insect influenced the transmission rate, and this suggests that where a mass-action phenomenon occurs it may be due to insects disturbing each other's feeding when present in large numbers.

Pseudococcus njalensis is a particularly suitable vector for the study of any such mass-action; its stylets are long and the feeding insects tend to cluster together. In histological preparations the stylet tracks were seen frequently to run close together in the plant tissues. The cacao embryos used for test feeding were small, so that they also favoured the demonstration of mass-action.

The probability of infection by one insect calculated from sixty trials of single insects was 0·1 for both viruses 1 A and 1 M. Call this probability p and let $q = 1 - p$; then the probability of infection by n insects was $(1 - q^n)$, and therefore the probable

number of test plants infected by any given number of insects can be calculated. The calculated and the observed numbers infected out of sixty test plants for groups of five to thirty insects are given in Table 3 for viruses 1 A and 1 M. With both viruses the probability of the Goodness of Fit being correct was between 0·8 and 0·9, so there was no evidence that the larger groups of insects gave an infection rate higher than would be expected if their inoculations were local and independent, i.e. there was no mass-action effect.

Table 3. *The observed and calculated numbers of test plants infected showing absence of 'mass-action' with* Theobroma *viruses* 1 *A and* 1 *M and* Pseudococcus njalensis

No. of insects	Probability of infection ($1-q^n$)	No. of infections out of sixty test plants				
		Calculated	Observed (M)	χ^2	Observed (A)	χ^2
5	0·4095	24·570	29	0·799	32	2·247
10	0·6513	39·078	36	0·242	42	0·219
15	0·7941	47·646	42	0·669	48	0·003
20	0·8786	52·716	48	0·422	54	0·031
25	0·9282	55·692	56	0·002	57	0·031
30	0·9576	57·456	55	0·105	56	0·004
				$\chi^2=2·239$		$\chi^2=2·534$

(2) *The effect of insect maturity on infection rate*

Although Posnette & Strickland (1948) suggested that the first and second instars of *Ps. njalensis* might be more efficient vectors than the older stages, their figures were inconclusive. Adults of *Ps. njalensis* were found to be less suitable for comparing with nymphs than those of *Ps. citri*, because the latter were easier to establish on leaves. A comparison of transmission by *Ps. citri* adults with that of first and second instar nymphs was made, using five insects (from the same virus source) to each of ten test plants, replicated ten times.

The difference between 47 % transmission by adults and 29 % by nymphs was significant at the 5 % point, showing that adults of *Ps. citri* were more efficient vectors and that the use of mixed instars would increase experimental error. Great care was taken to avoid any damage to the insects so that the difference was not due to injury of the nymphs.

(3) *The effect of age of virus source plant on infection rate*

Although uniform infection rates could usually be obtained when young seedlings were used as source plants, from older plants the results were often variable. The concentration or availability of the virus appeared to decline as the plant became older. This might be expected with viruses which cause dissimilar acute and chronic phases, but it was also noticed with virus 1 C, which does not (Posnette, 1947*a*). Few transmissions with insects were obtained from mature trees or from seedlings

which had been infected with virus 1 C for over a year, but once the virus had been transferred to seedlings, subsequent transmissions were readily obtained from them. The effect of the symptom pattern on transmission is shown in Table 4, and of leaf age in Table 5. These results show that immature leaves with the red-veining symptom provide the best virus source, and that slight differences in the maturity of the leaves of the source plants could lead to considerable variation in the infection rate.

TABLE 4. *Effect of leaf symptom of virus source on infection rate of virus 1 M using five insects per test plant*

Exp. no. ...	1	2	3	
	No. of infections out of ten test plants			
Symptom pattern				Total
Red veining	4	6	9	19
Total mosaic pattern	1	1	8	10
Preliminary pattern	1	1	7	9
Symptomless	0	3	0	3

TABLE 5. *Effect of age of leaf of virus source on infection rate of virus 1 M using five insects per plant*

Exp. no. ...	1	2	3	4	5	6	
Symptom pattern	No. of infections out of ten test plants						Total
(A) Red veins, unexpanded leaf	1	2	6	10	5	8	32
(B) Red veins, expanded leaf	4	4	5	9	8	7	37
(C) Total pattern, leaf soft	3	5	4	7	5	7	31
(D) Total pattern, leaf hard	0	1	1	1	3	3	9

(4) *Localization of the virus in the plant*

In several experiments when the insects were not randomized among the test plants transmission tended to occur in runs of test plants, followed by groups of uninfected plants, suggesting that virus concentration might vary in different parts of the source plant. This was investigated by colonizing separate parts of plants infected with virus 1 M and doing comparable transmission tests. Although normal infection rates were obtained from twenty-two feeding sites, no transmission was obtained from four sites (each tested by fifty vectors from random sources); three of these were petioles and the fourth was a stem. The probability that the lack of transmission by these colonies is due to chance is 0·005 for each of them. This suggests that systemic infection was incomplete.

(5) *Penetration time*

Most viruses whose vector relationships have been studied are transmitted either by aphids or by leaf-hoppers; their stylets penetrate the plant tissues rapidly and feeding can commence within a few minutes of the insect settling (Storey, 1938).

The time required for mealybugs to commence feeding is much longer because of the length and fineness of their stylets. Before the penetration time could be considered in feeding-time experiments the feeding mechanism of *Ps. njalensis* had to be studied. Carter's (1945) technique was used; 3 % agar with 3 % added glucose was poured in thin layers in Petri plates. After drying in a desiccator overnight, rings of agar were cut and sandwiched between a slide and a cover-glass, with first and second instar nymphs of *Ps. njalensis* imprisoned within the ring. The insects were examined under the low power of a microscope at various times after being placed in the dark, and the following account of the penetration process is pieced together from numerous observations. After the first 10 min. during which the mealybug nymphs wandered over the glass, they began to settle on the agar, particularly in corners and cracks. Some tested the substrate by means of the rostrum and its ring of terminal hairs. The labium was closely adpressed to the substrate and after a variable time the stylets protruded from the tip of the rostrum and moved, usually in a straight line, slowly into the agar. Viewed with the low power, this movement appeared slow and steady. Under the $\frac{1}{6}$ lens, however, the movement resolved itself into a series of to and fro thrusts. A droplet of saliva was secreted at or near the tip of the stylet tube. This viscous droplet appeared to be secreted under slight pressure ahead of the extended tip of the stylet tube, and after its secretion the stylets moved back a little and then thrust forward through the droplet to mould a tube.

The stylets showed not only an up and down movement during penetration but also a lateral motion at the tip. This lateral movement appeared to be imparted from the musculature of the buccal region but it was not possible to observe whether or not there were discrete movements of the components of the stylet tube. During the whole course of penetration by the stylets, the rostrum of the insect kept up a rapid pulsating movement. This could not be related to the movement at the tip of the stylet tube, because both the tip and base of the stylets could not be seen in the field of the microscope at one time. The rostrum certainly played a part in the extension of the stylets in gripping the base of the stylet tube between each thrust if it did not supply part at least of the motive power. When the stylets were completely withdrawn by the insect they left a salivary tube which had a laddered appearance under the low power; and under the high power it appeared to be made up of a string of salivary droplets penetrated by a central tube. The rate of movement of the stylets into the agar was on the average 0·5 μ/sec. for both adults and nymphs, although adults penetrated to a greater depth than did 'crawlers'. In one observation, where the stylets were measured at full extension, the average length of the stylets of eleven adults was 988 μ. The average length of the stylets of eleven first instar nymphs was 492 μ.

From these observations the actual penetration time for nymphs could be calculated at about 16 min. It seems unlikely that the speed of penetration in agar was slower than that in the plant. In sections of leaves, the stylet tracks stained with safranin

(Smith, 1926) could be seen entering the vascular tissue, but it was uncertain whether they ended in the xylem or the phloem. Although feeding is probably from the vascular tissue we do not know whether penetration this far is necessary, before virus transmission can occur.

Vector-virus relationships

(1) *Duration of test feed and the infection rate*

The effect of increasing the time of test feeding on the infection rate with virus 1 M was first investigated, allowing *Ps. njalensis* nymphs (reared on the source plants) test feeds of 1, 2, 4 and 12 hr. There were ten test plants with five insects each in each treatment replicated six times. Infection rate varied only between 32 and 40 %; differences between treatments were not significant.

As no increase in the infectivity of the insects occurred with test-feeding periods greater than 1 hr., shorter feeding times were investigated, ten insects per test plant were used, those from different source plants being equally distributed between the treatments with six replications as before. The results for feeding times between 10 and 60 min. are given in Table 6; the data are best fitted by a linear regression of formula $Y = 3{\cdot}805 + 1{\cdot}27(x - \bar{x})$. Infection first occurred at 20 min. when full penetration might be expected, and reached a maximum at 50 min. There was no indication of a latent period.

Table 6. *The effect of test-feeding time on infection rate with virus 1 M (ten insects per plant)*

Exp. no. ...	1	2	3	4	5	6		
Time of test feeding (min.)	No. of infections out of ten test plants						Total	Mean
10	0	0	0	0	0	0	0	0
20	1	1	1	1	3	2	9	1·5
30	2	5	5	3	3	6	24	4·0
40	7	4	7	3	5	6	32	5·3
50	9	2	6	7	8	5	37	6·2
60	7	4	8	6	5	5	35	5·8

(2) *Persistence of the virus during test feeding*

Posnette & Strickland (1948) showed that virus 1 A did not persist in the vector after a 3 hr. test feed. Further experiments with transfers to fresh test plants at ½ hr. intervals have invariably given a very low infection rate with both virus 1 A and 1 M. This was due probably to the long penetration time and to the difficulty of repeatedly extracting the stylets undamaged. On only three occasions in our experiments has an insect infected two plants in a series with virus 1 M—once was after an interval of ½ hr. and twice when they were the first and second plants in a series.

There seems to be little doubt that virus 1 M should be classified as non-persistent in the vector, although, as discussed later in this paper, persistence up to 36 hr. has been demonstrated in insects starved after the infection feed.

(3) *The effect of preliminary starvation and the duration of infection feed on the infection rate*

Experiments with infection feeds ranging from 3 min. to 3 hr. gave very low rates of transmission, and although infectivity increased with feeding time, the increase was slow even when the insects were starved before use. Intervals of 2 hr. were therefore adopted for the study of the effect of increasing the infection feed.

The insects were bred from intentionally mixed and randomized colonies, and those from each source plant were randomized among the treatments. Starving insects were kept for 12 hr. in small weighing bottles, precautions being taken to maintain them at an even temperature and humidity. Infection feeding took place in the dark (except while the insects were being transferred) on uniform, recently infected, young seedlings. Dissected cacao beans were used as test plants with five insects to each. There were ten beans in each treatment with six replications.

The data obtained with virus 1 M transmitted from leaves is given in Table 7, together with the calculated figures for the cubic regression equation best fitting the 'starved' data, and the linear regression equation fitting the 'unstarved' data. The infection rate rises more rapidly with the pre-starved than with the unstarved insects, and reaches a peak at 10 hr. With pre-starved insects there was a significant drop in infection rate at 12 hr.

Table 7. *Effect of preliminary starvation* (12 *hr.*) *and duration of infection feed* (*on leaves*) *on infection rate*

Infection feeding time on plant (hr.)	Mean no. of infections out of ten test plants			
	Pre-starved		Unstarved	
	Actual	Calculated	Actual	Calculated
2	2·17	2·298	0·16	0·89
4	2·67	2·28	1·83	1·73
6	3·83	4·06	3·50	2·58
8	5·67	5·99	3·33	3·42
10	6·83	6·40	4·83	4·27
12	3·50	3·638	4·33	5·12

Difference significance at 1% = 2·63.

It was expected that starved insects would settle to feed on the source plant more rapidly than unstarved insects, and that this would influence infection rates obtained with short infection feeds. Since it was impossible to see whether the stylets of *Ps. njalensis* had penetrated the plant, an estimate of the proportion of insects settled and feeding was obtained by carefully touching the insects with a fine brush.

This disturbed insects which had just begun to penetrate, and the number of insects whose stylets were firmly fixed was taken as the number of feeding insects.

The settling rates of the insects on the source plants in the experiment summarized in Table 7 are given in Table 8.

TABLE 8. *The effect of preliminary starvation on the settling rate of* Pseudococcus njalensis *nymphs on leaves*

Time on plant (hr.)	No. of insects settled out of fifty (mean of six experiments)			
	Pre-starved		Not starved	
	Actual	Calculated	Actual	Calculated
2	18·50	18·62	6·66	6·34
4	18·33	19·95	10·66	11·73
6	23·16	21·29	16·33	15·50
8	23·50	22·61	17·50	17·34
10	24·66	23·94	16·33	17·06
12	21·94	25·28	14·83	14·47

The chief difference in the behaviour of the two sets of insects occurred in the first 2 hr. Between the second and the twelfth hour the number of starved insects actually feeding showed a slight increase, represented by a regression coefficient of 1·33. The unstarved insects settled much more slowly; about one-third of the insects which finally settled did so within the first 2 hr., and there was a sharp rise in the numbers settled until the sixth hour. These figures are best fitted by the polynomial regression equation $Y = 13{\cdot}722 + 0{\cdot}843\xi_1 - 0{\cdot}652\xi_2 - 0{\cdot}019\xi_3$. When the smoothed curves from Tables 7 and 8 are compared in Fig. 1, it is apparent that the difference in the effect of time of infection feeding was not primarily a reflexion of the settling rate; although the initial lag in time between the curve of infectivity of pre-starved and unstarved insects probably reflects the similar lag in settling between the two sets of insects, the infection rate with starved insects (Table 7) continued to rise after the settling rate had become nearly stable.

A direct comparison of the infectivity of starved and unstarved insects was rendered difficult by the difference in their settling rates, and it was thought that this difference might be minimized by the use of cotyledons as a source of virus. It was known from the test-feeding experiments that a high proportion of unstarved insects had settled and fed on cotyledons within 30 min. (The quality of cotyledons which induces quick settling is the number of folds and crevices in which the mealybugs prefer to settle.)

To allow further development of the curves of infection rate with duration of infection feed, the experiments were extended to 18 hr. As before, ten test plants, each with five insects, were used per treatment, but five instead of six replications. The results are given in Table 9.

The first figure in column 4 of Table 9 is deceptively low, being weighted by a zero term. Allowing for this, comparison with Table 8 shows that the difference

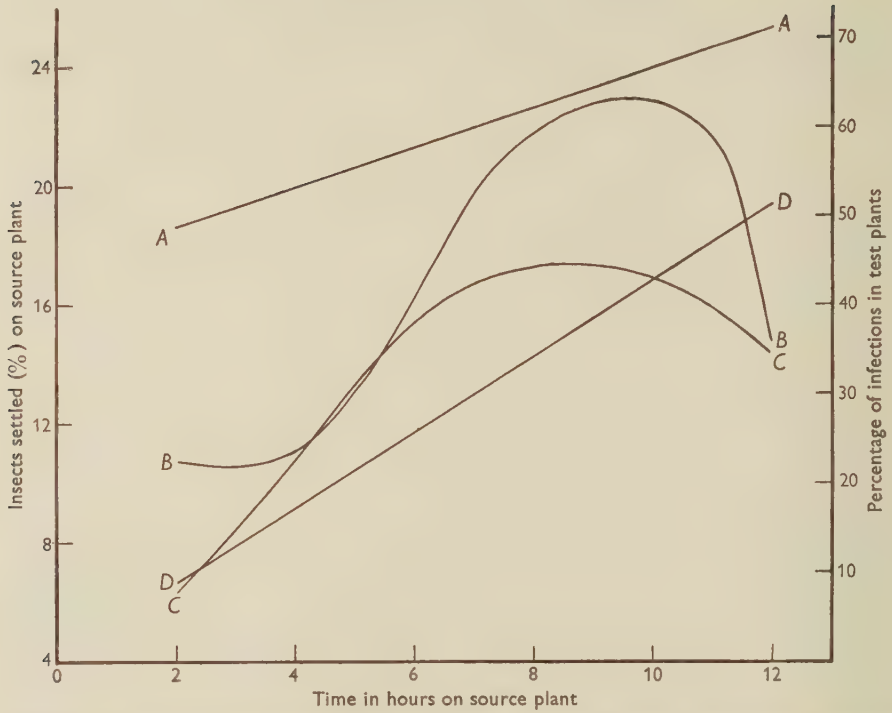

Fig. 1. Graph showing the relation between settling time, infection feeding and infection rate. *AA*, represents the settling rate for pre-starved insects placed on leaves for infection feeding; *BB*, represents the infection rate for these insects; *CC*, represents the settling rate for unstarved insects placed on leaves for infection feeding; *DD*, represents the infection rate for these insects.

TABLE 9. *Effect of preliminary starvation* (12 *hr.*) *and duration of infection feed* (*on cotyledons*) *on settling and infection rate*

	Pre-starved insects			Unstarved insects		
Hours on source plant	Mean no. plants infected out of ten		Mean no. insects settled out of fifty	Mean no. plants infected out of ten		Mean no. insects settled out of fifty
	Actual	Calculated		Actual	Calculated	
2	0·4	1·29	5·58	0·2	0·90	13·20
4	1·0	1·65	13·56	0·6	1·17	11·76
6	0·8	2·00	15·60	1·8	1·44	18·72
8	2·6	2·37	24·36	0·5	1·71	19·68
10	3·0	2·73	24·00	1·0	1·98	19·44
12	1·6	3·09	21·16	1·8	2·25	23·28
14	1·6	3·45	25·56	2·8	2·52	21·84
16	4·4	3·81	26·76	1·8	2·79	19·68
18	3·2	4·17	25·20	2·6	3·06	22·56

between starved and unstarved insects settling on cotyledons was less than the difference on leaves. Contrary to expectations from the results of test-feeding experiments, both starved and unstarved insects showed an increase in settling *after* the first 2 hr. on the cotyledon.

The infection rate with unstarved insects rises up to 18 hr. and might have continued to rise had the experiment been continued with longer feeding periods. There was an indication between 10 and 12 hr. of a drop in the infectivity of starved insects similar to that which occurred with leaf-infection feeding, but the figures were too low for statistical significance. It is interesting to note, however, that infection rate was largely independent of settling rate when the two sets of insects are compared. For instance, 48 % of the starved insects settled at 10 hr. gave 30 % infection, while 47 % of the unstarved insects settled at 12 hr. gave only 18 % infection.

(4) *Post-infection starvation and infection rate*

In considering the possible effects of digestion in causing virus inactivation in the insect after 10 hr. feeding it was desirable to discover how long the virus was retained by insects starved after the infection feed. Table 10 shows the effect of continuous starvation on the infectivity of a population of fully fed insects (*Ps. njalensis*) which were not starved before infection feeding with virus 1 M.

TABLE 10. *Virus persistence in insects starved after the infection feed*

Hr. starved	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	48	74
Exp. no.	No. of plants infected out of ten by five insects per plant																						
1	8	—	5	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	-	-	-	—
2	4	—	6	1	—	—	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
3	8	—	—	10	—	—	7	—	—	1	—	—	1	—	—	-	-	-	—	-		-	-
4	1	—	—	3	—	—	1	—	—	1	—	—	—	—	—	1	—	—	0	—	—	0	0
5	4	3	6	4	2	5	5	1	3	2	—	—	3	—	—	1	-	-	-	-	-	—	—
6	8	—	—	—	—	—	—	—	—	—	—	—	2	—	—	0	—	—	—	—	—	—	—
7	2	6	4	4	5	6	2	2	2	3	8	2	0	3	4	1	1	1	0	0	0	—	—

The results of starvation are not consistent, and the insects seemed to become more efficient retainers of the virus in the later experiments. The method of keeping the insects in a healthy condition was improved during the first three experiments. The last three were all done by an improved method in which the insects were transferred from the source plants to weighing bottles which stood in a dish of shallow water and covered with a bell jar. The infectivity of the starving insects appeared to decline after about 20 hr. In similar experiments with virus 1 A and *Ps. citri* there was no decline in infection rate up to 18 hr. post-infection starvation, but at this time infectivity declined and no transmission occurred after 36 hr. starvation.

(5) *Separation of viruses from a complex*

When cacao plants were infected with both virus 1 A and virus 1 M they were not mutually antagonistic. The symptoms caused by each virus occurred independently on some leaves, but periodically leaves were formed with a characteristic 'complex' mosaic quite unlike the symptoms caused by either virus. There are no differences in vector relationships which could be used to separate these viruses with *Ps. njalensis*, although they were separable by different vector species (Posnette, 1950).

To find whether the viruses were so intimately associated in the plant that they would be transmitted simultaneously by one insect, as in tobacco rosette viruses (Smith, 1946), four plants infected with both were colonized with *Ps. njalensis*. The insects were transferred one to each test plant. Table 11 shows that the plants differed greatly in virus availability; one plant gave only virus 1 A transmissions and another only virus 1 M, but from two plants both viruses were transmitted. It is clear, however, that the viruses were seldom so closely associated in the plant that they were available to a single vector, and this emphasizes what has already been said about virus localization.

Table 11. *Transmission by single insects from virus 1 A + 1 M complex*

Plant no.	Symptoms on test plants			
	1 A	1 M	1 A + 1 M	0
1	6	—	—	35
2	—	6	—	28
3	4	32	7	82
4	3	7	5	62

Discussion

The results given on the vector-virus relationships show that virus 1 M does not fit precisely into either of the two main groups postulated by Watson & Roberts (1939): (*a*) *persistent viruses* which survive for long periods in their insect vectors, which have a non-infective latent period, are generally not sap transmissible and do not respond to preliminary starvation of the insect; and (*b*) *non-persistent viruses* which survive in their insect vectors for a relatively short period, have no latent period, are often sap-transmissible and temporarily respond to preliminary starvation of the insect by a greatly increased transmission rate. Most of the non-persistent viruses are transmitted by aphids while the persistent viruses are transmitted by a wide variety of hemipterous insects including the aphids. Two viruses have been described, the transmission behaviour of which resembles in some respects that of *Theobroma* virus 1 M. These are sugar-beet yellows virus (Watson, 1940, 1946) and dandelion yellows virus (Kassanis, 1947), which both show an increase in infection rate with increase in test-feeding and infection-feeding time of their vectors.

With *Theobroma* virus 1 M and its vector, an increase in test-feeding time from 20 to 60 min. caused a linear increase in infection rate. No increase for periods beyond 1 hr. was found, but a slight increase might have occurred without detection. Except for this failure to demonstrate an effect of test-feeding times longer than 1 hr., our results agree with those obtained by Watson for sugar-beet yellows virus and test feeding with *Myzus persicae* (Sulz.). She found that with increasing time on the test plants there was a rapid increase in the number of transmissions during the first 3 hr. and thereafter a slight though appreciable increase up to 20 hr. With aphids the penetration time is short; good penetration of parenchyma tissue to a depth of about 100 μ occurs in 5 min. by *M. persicae* (Roberts, 1940), although the time taken to reach the phloem is much greater, 77 % of the aphids having reached the phloem in 24 hr. With *Pseudococcus njalensis* the penetration time (i.e. the full extension of the stylets) is probably about 20 min., although we have no information as to whether penetration to the phloem takes place. Such a penetration time, together with an increase in the proportion of insects ready to feed during the first 40 min. would be sufficient to explain the test-feeding results obtained.

The efficiency of *Ps. njalensis* as a vector of *Theobroma* virus 1 M was increased by longer feeding times up to 10 hr. on the infected source plant. This compares with Kassanis's results with dandelion yellows virus where an increase in infectivity was found with increased length of infection-feeding time up to 1 day after the commencement of feeding. Kassanis's results also showed a decrease in infectivity on the third day and this might be compared with the decrease after 12 hr. feeding by the pre-starved mealybugs; Kassanis's insects were not, however, starved. The effect of infection-feeding time on the transmission of *Theobroma* virus 1 M can be compared with the results obtained by Watson with sugar-beet yellows virus where increased infectivity was obtained with infection feeding up to 24 hr. *Theobroma* virus 1 M was non-persistent in the vector, however, whereas the sugar-beet yellows virus was persistent. Watson would class both sugar-beet yellows virus and dandelion yellows virus as persistent viruses because their vectors did not respond to preliminary starvation, but Kassanis did not think this justified. He thought that the very low concentration of dandelion yellows virus in the plant necessitated a long infection-feeding time and that this masked any response to starvation in the insect. In spite of the long feeding time required, *Theobroma* virus 1 M did respond to pre-infection feeding starvation and only part of this response can be related to the settling rate and possibly to the appetite of the insects. We do not know, however, whether the drop in infectivity at 12 hr. from the peak reached at 10 hr. with the pre-starved insects would occur later, in those not pre-starved, had the experiment continued beyond 18 hr. The main question at issue is whether this drop in infectivity at 12 hr. bears any relation to the drop in infectivity after a few minutes feeding shown by the non-persistent aphid transmitted viruses.

Watson & Roberts (1939) postulated that the drop in infectivity in the non-persistent viruses was caused by the secretion of an inactivating substance by the

insect's gut shortly after the stimulus of feeding has taken place. On this hypothesis the virus taken in during the first few minutes was not affected by the inactivating or destroying substance and during this period the infectivity of the insect was at a high level and proportional to the length of time of infection feeding. After a few minutes' feeding the inactivating substance operated, and more virus was inactivated than was taken in; this effect was continued for several hours after the commencement of infection feeding. In pre-starved mealybugs fed on infected leaves there was a sudden drop in infectivity after 12 hr. infection feeding. If this drop in infectivity was due to the secretion of some substance from the insect's gut, it seems strange that 12 hr. elapsed before it was secreted, although it is possible that the substance was part of the later processes of digestion only. Another explanation is that although an inactivating substance was present, it was secreted continually by the gut of the feeding insect and was continually inactivating the virus, the uptake of which exceeded the inactivation so that the virus slowly accumulated until the insect was replete; when the insect stopped feeding, inactivation became the dominant process. This theory is favoured by the fact that inactivation occurred at the peak of the curve and not after it had levelled out. If this is the explanation of inactivation in pre-starved insects, then the virus in the unstarved insects should have behaved in a similar fashion. In the aphids the inactivating substance was apparently an immediate response to the presence of food in the gut; in *Ps. njalensis* inactivation may have occurred at repletion in both starved and unstarved insects, but showed in the starved insects because of their physiological uniformity and because they start and finish feeding earlier. If this should be so, then *Theobroma* virus 1 M would not fall strictly into the non-persistent category although showing an apparent response to pre-infection starvation. The basic phenomenon in both the non-persistent viruses and *Theobroma* virus 1 M would, however, be the same and the pattern of rise and fall would be secondarily and quite differently determined by the feeding behaviour of the insects, by the differing concentrations of active virus and inactivator and by the speed of intake of the virus by the aphid and mealybug respectively.

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VIRUS DISEASES OF CACAO IN WEST AFRICA

VII. VIRUS TRANSMISSION BY DIFFERENT VECTOR SPECIES

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Thirteen species in the Coccoidea were tested as vectors of seventeen cacao viruses or virus strains; in addition to the vectors previously recorded, the following species transmitted some of the viruses: *Paraputo ritchiei* Laing, *Phenacoccus* sp. H 6418 (? sp.nov.) *Pseudococcus bukobensis* Laing, *Ps. brevipes* (Ckll.), *Pseudococcus* sp. near *celtis* Strickland, *Pseudococcus* sp. near *gahani* Green, *Ps. longispinus* T.-T. and *Pseudococcus* sp. near *masakensis* James. The viruses transmitted by *Ps. longispinus* were not usually transmitted by *Ferrisia virgata* (Ckll.), but there was no other virus-vector specificity.

Earlier work showed that some cacao viruses could be transmitted by *Ferrisia virgata* (Ckll.), *Pseudococcus njalensis* Laing and *Ps. citri* (Risso). Box (1945) suggested that there was some degree of specificity between these vectors and the viruses they transmit. The investigations reported here were made partly to find out whether cacao viruses and virus strains could be classified by their vectors. A knowledge of what insects can transmit cacao viruses is also important in investigating the range of wild plants acting as virus hosts, for a virus might be transmitted from alternative host plants to cacao by a vector species different from that mainly responsible for spread within the crop.

The vector species

Concurrently with this investigation, Strickland (1947, 1948) was studying the biology and bionomics of the Coccoidea attacking cacao on the Gold Coast. He found that *Ps. njalensis* is the most common species of the Pseudococcidae on cacao, with *Ps. citri* the second. *Ferrisia virgata* has been taken occasionally on cacao, but is common only as a garden pest. *Pseudococcus bukobensis* Laing has been found only once in large numbers on cacao, but was reared without difficulty on cacao in the greenhouse. *Ps. concavocerarii* James was seldom found and did not breed readily on cacao seedlings; most of the specimens collected in the field were taken from cacao pods.

Ps. brevipes (Ckll.) was never found on cacao in West Africa. When transferred to cacao from pineapple on which it is a common pest, insects survived for only a few days.

Ps. longispinus T.-T. was seldom found on cacao but is a common garden pest at Tafo on Jasmine, Aristolochia, and Maiden-hair fern (*Adiantum* sp.).

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Pseudococcus sp. near *gahani* Green (H 6421) has been collected twice and bred readily on cacao. The identity of this species is in doubt. Dr W. J. Hall (in litt.) states: '...In my view it comes closest to *P. gahani* Green, but differs from that species in having the trilocular pores on the anal cerarii rather more closely clustered round the cerariian spines. Also *gahani* has not "very long anal filaments" such as you describe.'

Pseudococcus sp. near *celtis* Strickland (H 6424) was collected from cacao near Asalu, Nigeria, where it was the commonest mealybug species. Of this species Dr Hall writes: 'I would not refer this to...*P. celtis*. Admittedly it comes very close to it, and it may be that later you will collect intermediate forms, but there are distinct differences between it and *celtis*....'

Pseudococcus sp. near *masakensis* James (H 6090) was collected only in the Western Province of the Gold Coast from *Cola chlamydantha* trees from which it transmitted the local virus to cacao. Dr Hall states: 'It is not unlike *P. masakensis* James, from Uganda, but the cerarii in particular are different.'

Phenacoccus madeiriensis Green is common as a garden pest both in Accra and Ibadan, but has not been taken from cacao, and artificial infestations on cacao did not thrive. No virus transmission was obtained with this species.

Phenacoccus sp. (H 6418). This species is of special interest because it causes distortion of young leaf and stem tissues of cacao. This toxic effect bears only a slight resemblance to known virus diseases, but the leaf crinkle, red blotching on the young leaves and the stem thickening might be mistaken for virus symptoms, and the stem thickening with enlarged stipules might be confused with Witches' Broom (*Marasmius perniciosus* Stahel). *Phenacoccus* sp. (H 6418) is rare at Tafo, but a similar species (*Phenacoccus* sp. H 6435) was found (A. F. P.) causing considerable damage to cacao in Zanzibar.

Paraputo ritchiei Laing. This species was collected from cacao on several occasions. It was usually found in cracks in the bark, and because attempts to establish it on young cacao seedlings have failed, it has been possible to test this species with only one group of viruses. As with *Pseudococcus* sp. (H 6090), virus transmissions to cacao were obtained from infected *Cola chlamydantha* trees using nymphs of *Paraputo ritchiei* (Posnette, Robertson & Todd, 1950).

It is admittedly unsatisfactory to have to refer to several of the vector species concerned in this paper by means of code figures, but specimens of all such species are in the Coccid collection of the West African Cacao Research Institute, and at the Commonwealth Institute of Entomology in London. Consequently, it is hoped that no confusion will arise over the identities of these species in the future.

Method

Seventeen viruses, some of them probably strains, were selected as representative of the wide range known from the Gold Coast, Nigeria and the Ivory Coast. The symptoms caused by only six of these viruses have been described in detail (Mangenot,

Alibert & Basset, 1946; Posnette, 1947), but the characteristic effects of the others are mentioned in the West African Cacao Research Institute Annual Reports.

The first tests were usually done by feeding *Pseudococcus njalensis* on graft-infected cacao seedlings and transferring the vectors to the cotyledons of fresh cacao beans (Posnette & Strickland, 1948). In subsequent tests, the infection feeding was on cacao seedlings which had been infected in this way, so that each vector species was tested with a virus which had previously been transmitted by a different species. This method was adopted to avoid the possibility of different vectors transmitting different components of a virus complex, but in fact no such resolution of complexes occurred except where the complex had been intentionally formed by double inoculation. Such 'artificial' complexes were used for confirming the specificity of certain virus-vector relationships, transmission of only one *consistently* from a plant infected with two unrelated viruses being regarded as corroborative evidence that the vector was not capable of transmitting the second (Posnette & Robertson, 1950).

The insects were collected from the field and some mounted for identification. If enough were available, they were transferred direct to infected cacao seedings, but otherwise an attempt was made to breed them on the cotyledons of healthy cacao seedlings in an insect-proof house. As far as possible, different species were kept in separate houses.

The infection-feeding time was at least 7 days because the insects were given time to multiply on the source plants before they were transferred singly with a brush at the rate of ten per test plant, on which the test-feeding period was 24 hr. (As *Ps. brevipes* did not become established on cacao, its infection-feeding time was reduced to 24 hr.)

Results

The results of these experiments are summarized in Table 1. Numerous experiments have been made with viruses 1 A, 1 C and 1 M and *Ps. njalensis*, *Ps. citri* and *Ferrisia virgata* in the course of earlier investigations (Posnette & Strickland, 1948; Posnette & Robertson, 1950), and only a few representative results are included. Experiments with other species were limited by the number of insects and the rarer species could not be tested adequately with all the viruses.

Pseudococcus njalensis, *Ps. citri*, *Ps. bukobensis* and *Pseudococcus* sp. near *celtis* (H6424) have transmitted all but one of the viruses with which they have been fully tested. *Paraputo ritchiei*, *Phenacoccus* sp. (6418), *Pseudococcus brevipes*, *Pseudococcus* sp. near *gahani*, and *Pseudococcus* sp. near *masakensis* have each transmitted one or more viruses. The chief interest in the results lies, however, in the differences between *Ferrisia virgata* and *Pseudococcus longispinus* in their ability to transmit different viruses. *Ferrisia virgata* has consistently failed to transmit virus 1 C, which has been transmitted by *Pseudococcus longispinus*, although at a low rate. *Ps. longispinus* also transmitted virus 1 M which, except in one experiment, was not transmitted by *Ferrisia virgata*.

TABLE 1. *Results of some transmission tests with seventeen viruses and thirteen species of Pseudococcidae (ten insects per plant)*

Virus 1 / Vector species	Gold Coast								Nigeria							Ivory Coast	
	A	C	D	F_1	H	J	K	M	N	O_1	O_2	O_3	P	R	S	X	Y
Pseudococcus njalensis	5/5	4/10	7/20	5/10	5/10	4/10	12/20	5/5	9/20	16/30	6/30	5/5	4/5	2/10	0/20	2/5	5/10
Ps. citri	3/5	6/10	2/20	1/1	2/10	5/5	27/30	8/10	.	5/5	9/10	4/10	5/5	5/8	0/27	+	5/10
Ps. bukobensis	7/10	.	.	1/8	2/2	2/5	1/2	.	1/10	2/15	2/10	1/3	4/5	2/10	.	.	0/10
Pseudococcus sp. near *celtis*	10/10	5/20	.	4/5	19/20	.	4/5	.	2/3	3/3	.	.	7/15	15/15	.	0/64	19/20	.
Pseudococcus sp. near *gahani*	3/10	0/10	.	.	.	.	.	4/20	.	.	.	.	5/9	.	.	.	.
Ps. longispinus	0/10 0/60	2/20 5/60	0/20 0/20	0/2	0/5	0/5	0/18	26/60	0/4	0/20 0/20	0/19	0/14	0/27	0/8	.	0/10	0/20
Ferrisia virgata	5/10	0/10 0/60	0/10 0/20	7/15	13/15	.	12/20	0/86 13/60	12/50	1/10	5/10	5/10	5/5	0/50	0/17	+	5/20

The following results were obtained with other species:

Ps. concavocerarii with virus 1 P, 0/18.
Ps. sp. near *masakensis* (H 6090) with virus 1 (F), 6/7.
Ps. madeiriensis with virus 1 A, 0/46; with virus 1 M, 0/10.
Ps. brevipes with virus 1 A, 1/15; with virus 1 C, 1/8.
Phenacoccus sp. H 6418 with virus 1 M, 2/2.
Paraputo ritchiei with virus 1 (F), 2/6.

Key to type localities of the viruses:

A = New Juaben	H = Dawa	N = Akanran	R = Alaparun
C = Kpeve	J = Bosomtwe	O_1, O_2 = Olanla	S = Asalu
D = Nkawkaw	K = Kononga	O_3 = Ilesha	X = Kongodia
F = Wiawso	M = Mampong	P = Offa Igbo	Y = Yakasse

N.B. The grouping of these viruses with distinguishing letters under *Theobroma* virus 1 is a temporary expedient and does not indicate strain relationships.

When fed on plants infected with virus 1 A and 1 M, *F. virgata* transmitted only virus 1 A. We do not understand why *F. virgata* transmitted virus 1 M in one experiment; it is possible that a biological race was involved as with *Pseudococcus citri* discussed below, but no transmission was obtained when the experiment was repeated using insects from the same source. The possibility of contamination with another vector species cannot be eliminated, but seems improbable because of the precautions taken and the distinctive appearance of *Ferrisia virgata*.

The difference between *F. virgata* and *Pseudococcus longispinus* as vectors of viruses 1 C and 1 M is emphasized by their different behaviour as vectors of other viruses. *Ps. longispinus* failed to transmit all the other viruses, whereas *Ferrisia virgata* transmitted all except 1 D, 1 R and 1 S. When *F. virgata* was fed on a plant infected with both 1 R and 1 A, it transmitted only the latter.

The only virus for which no vector was found is that from Asalu (Nigeria). A collection made in this outbreak yielded *Pseudococcus* sp. near *celtis* (H 6424), but although this species was an efficient vector of the other cacao viruses, it failed to transmit the Asalu virus (1 S) from graft-infected plants. This virus, the symptoms of which resemble those reported from Venezuela (Posnette & Palma, 1944), does not cause swellings; its leaf symptoms differ from those caused by other cacao viruses in West Africa in the almost complete absence of red pigment from the young leaf, and the regular arrangement of small clearings along the midrib and secondary veins. When symptoms are severe, these veins are drawn towards the midrib causing distortion like that associated with the nutritional disorder known as 'sickle-leaf' and unlike the interveinal crinkling with viruses 1 C and 1 J.

Biological races of vectors

The failure to obtain transmissions in certain experiments suggested that strains of mealybugs unable to transmit might occur within the species. Ten gravid females of *Ps. njalensis* were collected from widely separated points within a radius of about 1 mile of the laboratory at Tafo. Their progeny were reared separately on isolated cacao plants in insect-proof greenhouses. When tested by the standard technique, each of the ten 'strains' transmitted virus 1 M and there were no striking differences in the infection rates. Although this is not proof of the absence of non-transmitting strains of *Ps. njalensis* it suggests that they are uncommon in the Tafo district.

Non-transmitting strains of *Ps. citri* do occur, however. Evidence of this was obtained using virus 1 A, but some isolates were lost through parasitism before the experiments could be concluded. Experiments with virus 1 C showed (Table 2) that one strain of *Ps. citri*, which was cultured from a single gravid female collected at Tafo, consistently failed to transmit under conditions suitable for transmission by the mixed progeny of five female *Ps. citri* collected at Kpeve (the locality of the only known outbreak of virus 1 C). Mr A. H. Strickland confirmed that these types were within the normal range of variation of *Ps. citri* as at present recognized in West Africa.

Table 2. *Comparison of transmitting and non-transmitting strains of* Pseudococcus citri (*five insects per plant*)

Exp. no.	Infection rate with	
	Tafo isolate	Kpeve isolate
1	0/5	4/5
2	0/10	3/10
3	0/10	3/10
4	0/10	0/10
5	0/10	2/10
6	0/10	4/10
Total	0/55	16/55

Discussion

Three considerations of practical importance arise from these results. The first is that control measures directed against the vectors should not be specifically against the common species, *Ps. njalensis*, but rather against the Pseudococcidae as a group. The second, which stresses the importance of controlling outbreaks of the 'mild' viruses, is that complexes formed by plants becoming infected with two or more viruses would spread as a complex, because the common vectors would transmit both viruses simultaneously. The third consideration is the classification of cacao viruses, the steadily increasing number of which demands some system of rational grouping to avoid confusion.

If the cacao viruses are regarded as one group, these experiments show that nine species of *Pseudococcus* are vectors; of those tested, only *Ps. madeiriensis* and *Ps. concavocerarii* failed to transmit. The cacao viruses in Trinidad are also transmitted by *Ps. citri*, *Ps. brevipes* and *Ferrisia virgata* (Kirkpatrick, 1950), but no other plant viruses with mealybug vectors are known. Transmissions by *Paraputo ritchiei* and *Phenacoccus* sp. H 6418 show that the vector range is not confined to the genus *Pseudococcus*; indeed, any genus in the Pseudococcidae must be suspect until proved not to be a vector. Those which are sufficiently common to affect spread within the crop have been tested, but other genera such as *Tylococcus*, *Formicoccus* and *Farinococcus* may start outbreaks by transmitting viruses from wild host plants such as *Ceiba pentandra* on which the common vectors are less prevalent than on cacao (Posnette *et al.* 1950).

It is clear from Table 1 that the cacao viruses (except 1 S from Asalu) can be transmitted by any one of a group of vectors which includes *Pseudococcus bukobensis*, *Pseudococcus* sp. near *celtis*, *Ps. citri*, *Ps. njalensis* and probably *Ps. brevipes*. The viruses transmitted by this group are not all closely related, judging by cross-immunity tests (Crowdy & Posnette, 1947; Posnette & Todd, unpubl.). When tested by graft inoculation, each of the viruses 1 H, 1 K and 1 X protected plants against systemic infection by virus 1 A, and they are probably related strains. Similarly, the three Olanla and Ilesha viruses are probably strains of a different virus. There was, however, no mutual antagonism between other combinations of the viruses.

It is not unusual for a virus to be transmitted by several vector species (cf. Severin, 1945), and the most interesting fact in Table 1 is that if the viruses are grouped into those which are and those which are not transmitted by *Ferrisia virgata*, the only viruses transmitted by *Pseudococcus longispinus* fall into the latter group. Three viruses, 1 C, 1 D and 1 R, which *Ferrisia virgata* failed to transmit all cause an intense red mottle on the young leaves of cacao. Viruses 1 C and 1 R do not cause stem swellings and 1 D causes only slight swellings occasionally. But virus 1 M, which also was not transmitted by *F. virgata*, causes red vein-banding of the virus 1 A type, and large swellings, and appears to be unrelated to the virus 1 C group; yet only viruses 1 C and 1 M were transmitted by *Pseudococcus longispinus*.

Symptoms are notoriously unreliable for indicating virus strain relationships, and vectors may be equally unsuitable. For example, Bawden & Kassanis (1947) found that the vectors of potato virus *Y* would not transmit the related virus *C*. It is interesting to note that the differences between the cacao viruses as regards the vectors *Ferrisia virgata* and *Pseudococcus longispinus* might support a classification based partly on symptoms, cross-immunity reactions and host range. No other characters are known; because repeated attempts at mechanical inoculation have failed, serological tests have not yet been attempted and dilution end-point and longevity *in vitro* are ruled out. The two viruses (1 C and 1 M) transmitted by *Ps. longispinus* also resemble each other and differ from the *Ferrisia virgata*-transmitted 1 A group in their host range (Posnette, *et al.* 1950). Although they do not

show antagonism in cross-protection tests, they may be more closely related to each other than to the other viruses used in the investigation.

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THE INFECTION OF PLANTS BY VIRUSES THROUGH ROOTS

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Roots of young tomato plants became infected when inoculated with tomato bushy stunt, tobacco mosaic, and potato *X* viruses. Root infections also occurred when these viruses were added to soil or culture solutions in which plants were growing.

The viruses were sometimes localized around their initial entry points in roots; sometimes they invaded the root system but not the shoots, and sometimes they produced full systemic infection of roots and shoots. In some experiments, but not all, systemic infections were more frequent when the upper tap root or superficial roots were inoculated than when fibrous roots were inoculated.

In both tomato and potato, virus *X* spread from diseased to healthy plants sharing the same culture solution, if their roots were in contact, but not otherwise. Infection of the roots of potato plants by inoculation, produced only one plant with virus-infected haulms, although several had infected tubers.

Attempts to infect the roots of plants with tobacco mosaic virus have been made by various workers and have given conflicting results. Allard (1917) found that it was inoculable to the roots of tobacco plants, but that the plants did not contract it from growing in infested soil. McKinney (1927) and Johnson (1937), however, both found that plants can contract this virus from infective material in the soil, although they differed as to the frequency with which this occurred. Price (1938) also infected tobacco and *Nicotiana glutinosa* plants by pouring infective sap on the soil in which they were growing, but found that the virus was restricted to the roots. Fulton (1941), like Allard (1917), found that roots were easily infected by direct inoculation, but that tobacco mosaic virus moved upward very slowly and that it rarely or only belatedly entered the stem or induced leaf symptoms. Few experiments have been made with other viruses, but Roberts (1948) showed that potato virus *X* spreads between the roots of infected and healthy tomato plants, and often causes full systemic infections. The experiments described herein were made to obtain further information on the behaviour of plants when infected through their roots.

Materials and methods

The experiments were made with glasshouse-grown tomato plants, var. Kondine Red, except for two with potato plants, var. Majestic. The strain of potato virus *X* used was X^Y, which causes a bright yellow mottle in Majestic potato, *Datura stramonium*, tobacco and tomato plants. Tests for this virus were made serologically and by inoculation to tobacco, var. White Burley, and *Datura stramonium*. Tests for tomato bushy stunt and tobacco mosaic viruses were made by inoculation to

Nicotiana glutinosa. Plants were grown either in unglazed pots containing potting compost, or in culture solution in various glass or glazed containers. The culture solution was that described by Arnon & Hoagland (1942–3). Except in one series of experiments, the culture solutions were not aerated, but the plants grew well for the required periods. Special methods employed for individual experiments are described later.

Results

Effect of adding infective sap to soil

Tomato plants were grown in 8 in. pots with precautions to prevent the aerial parts from touching the infested soil. Water-proof cellophane disks, with a diameter greater than that of the pots, were slit along a radius and a small hole was cut in the centre to accommodate the stem. The soil was covered with the disk, a 1 in. strip of pliable metal was bent into a loose ring around the base of the stem and the cellophane was 'pinned' to the soil by pressing downwards with the metal ring. The cellophane was folded to form a funnel-shaped structure whose edges were supported off the soil by short stakes. The plants could be watered at the sides of the pots, but no leaves could touch the soil, nor could the infective sap flow directly around the stem base. The infective sap was diluted and poured over the soil near the outside edge of the pots.

Table 1. *Effect of adding infective plant sap to soil*

Exp. no.	Duration of test	Virus	Quantity infective sap per pot	No. of plants tested	No. of plants infected		
					Leaves only	Roots only	Leaves and roots
D1	8 Jan.–5 Mar.	Bushy stunt	15 ml. at 1/6	12	0	1	5
	8 Jan.–11 Mar.	Potato virus *X*	10 ml. at 1/6	12	0	1	0
	8 Jan.–26 Feb.	Tobacco mosaic	24 ml. at 1/10	12	3*	1	3
	8 Jan.–27 Feb.	Tobacco mosaic	12 ml. at 1/20	12	0	0	0
D2	21 June–23 Aug.	Bushy stunt	20 ml. at 1/4	10	1	4	1
	21 June–1 Sept.	Potato virus *X*	20 ml. at 1/4	10	0	2	0
	21 June–24 Aug.	Tobacco mosaic	20 ml. at 1/4	10	1*	0	9
D3	29 Sept.–6 Dec.	Potato virus *X*	26 ml. at 1/4	15	0	0	1
	28 Sept.–6 Dec.	Tobacco mosaic	26 ml. at 1/4	15	0	9	1

* Leaves which gave one lesion only.

A summary of the results of three experiments is shown in Table 1. In the first, the plants were used when 4–6 in. high, and 3 weeks after the sap had been poured on to the soil four showed severe symptoms of bushy stunt. The leaves and roots of each plant were tested separately on *N. glutinosa* after 8 weeks, and these four plants gave numerous lesions; one which had not shown symptoms, gave fewer, and another gave only one lesion each from the root and leaf test. One plant had virus in its roots only. Comparable differences between the virus content of different plants was

observed several times when testing for bushy stunt and tobacco mosaic viruses, and the plants which at first sight appear in Table 1 as infected in the leaves only would probably have given isolated lesions from the roots also had more *N. glutinosa* leaves been inoculated. Often, however, plants infected in the roots only produced many lesions on the test plants, whereas inoculation from the corresponding leaves gave none. The production of only a few lesions presumably indicates that the tests were made soon after the viruses had begun to move from their initial entry points; at this time, when leaves are inoculated, virus often occurs in tissues remote from the site of infection while intervening tissues are virus-free (Samuel, 1934; Capoor, 1949). In the last experiment with tobacco mosaic, the virus was applied at two dilutions, 24 ml. of sap at 1/10, and 12 ml. at 1/20. Seven out of twelve plants became infected using the more concentrated sap, and none with the other.

Both bushy stunt and tobacco mosaic viruses were distributed differently through infected plants at different times of the year. In the first (winter-spring) test with bushy stunt six out of twelve plants became infected, of which five were infected in both roots and tops. In the second test, made in summer, six out of ten plants became infected but the virus was restricted to the roots of four. By contrast, tobacco mosaic entered the leaves of three out of the seven infected plants in the winter-spring test, nine out of ten in the summer test and one out of ten in the autumn-winter test.

Potato virus *X* was found in the roots of only four of the thirty-seven plants treated, much fewer than found previously (Roberts, 1948), when a larger quantity of infective sap was applied to the soil.

In the third experiment with virus *X* and tobacco mosaic, a preliminary leaf test of the tomato plants was made 18 days after sap was applied to the soil, and none was infected. All the plants were then cut back, and the newly developed shoots were tested again after 38 days. Tobacco mosaic was found in ten out of fifteen of the plants, but it had reached the leaves of only one; and only one plant was infected in the roots with potato virus *X*. Pruning did not encourage movement of virus from the roots, although it has been observed to do this when the pruned plants were also kept in the dark for some days (Bawden, 1943).

Direct inoculation of roots of plants growing in soil

Virus-free tubers of the potato variety Majestic were cut into suitable sizes and placed in moist sand for sprouts and roots to develop. Early in July, ten of the young plants were washed free from sand and their roots were dusted with celite and rubbed with sap containing virus *X*. The roots were thoroughly washed in running water and the plants set in soil in 10 in. pots. The tops of the young plants were tested serologically after 1 week and none reacted positively. Six weeks after inoculation one of the plants showed leaf symptoms and serological tests on the leaves confirmed that it, but no other plant, was infected. The roots and the tops were then tested separately, and virus *X* was found in the roots of nine out of the

ten plants inoculated, but it was found in the tops only of the one which had shown symptoms. The tubers were stored over winter, when they were tested for virus *X*. When possible, two of the largest tubers from each plant were tested individually and the other, often very small, tubers were pooled. Table 2 shows that infected tubers were set by plants which were infected only in the roots. This supports an earlier suggestion (Roberts, 1948) that potato virus *X* may spread between plants in the field without infecting the foliage or producing symptoms.

Table 2. *Distribution of virus in potato plants whose roots were mechanically inoculated*

Plant no.	Test on *D. stramonium* of		Test of eyes from tubers on *D. stramonium*		
	Tops	Roots	Tuber 1	Tuber 2	Remainder
1	−	+	+	+	+(3–8)*
2	−	+	−	−(2–4)	·
3	−	+	−	−	−(3–6)
4	−	+	−	−	−(3–4)
5	−	+	−	+(2–3)	·
6	+	+	+	+	·
7	−	+	−	−	−(3–4)
8	−	+	−	−	−(3–6)
9	−	−	−	−	−(3–5)
10	−	+	−	+	−(3–6)

* Numbers in parentheses indicate numbers of tubers included in test.
+ = positive reaction; − = negative.

Two groups, each of sixteen young tomato plants, were inoculated late in November, one with tobacco mosaic virus, and the other with a mixture of this virus and potato virus *X*. Each plant was removed from its pot and the soil gently crumbled from the roots, which were then washed. The average root system was a short (1–1½ in.), thick tap root just below soil level, which thinned rather abruptly, merging with a mass of adventitious fibrous roots. From the collar region of the plant the horizontal 'feeder' roots were beginning to develop. Treatments for both groups of plants were as follows: eight plants in each were inoculated with the fingers by rubbing the lower ends of the fibrous roots with a mixture of infective sap and celite, and the other eight by dipping the flattened end of a mounted needle into the inoculum and with this rubbing and slightly scratching a restricted area (about 0·5 sq.cm.) at the base of the thick portion of the tap root. Immediately after inoculation each root system was thoroughly washed in running water, and the plant re-potted. Care was taken to avoid accidental contamination of any part of the plant with the inoculum.

Five weeks after inoculation many of the plants showed symptoms, and leaves from the base and the top of each were tested on young tobacco plants. All sixteen plants inoculated with virus *X* alone were infected in both roots and tops.

Of the plants inoculated with both viruses in the fibrous roots, potato virus *X*

invaded the tops of seven of the eight plants treated, and tobacco mosaic virus only three; of the eight plants inoculated in the tap root, virus *X* reached the tops of all, and tobacco mosaic virus of six.

When the roots were tested, the eight tomato plants inoculated on their tap roots all produced many lesions on *N. glutinosa* and all contained virus *X*. The eight inoculated on the fibrous roots also all gave lesions on *N. glutinosa*, but variable numbers and some produced only a few, indicating a less general infection than in the plants inoculated in their tap roots; all eight also contained virus *X*.

Another similar test was made in spring with older tomatoes. Twelve plants in the first flower-bud stage were divided into two groups of six and inoculated in the tap roots respectively with tobacco mosaic and virus *X*. The leaves and roots were tested after 2 months, when five inoculated with tobacco mosaic virus were infected in roots and leaves, and the remaining plant only in the roots. The roots of this plant gave only three lesions on two *N. glutinosa* leaves, the others gave more than 100. The six plants inoculated with virus *X* were all infected in the roots, but only one in both roots and leaves.

Susceptibility to root infection of plants grown in culture solution

Tomato seedlings with the first true leaves just beginning to develop were set in glass containers in January 1949. These were bottles, 6·5 in. high, with mouths 1 in. in diameter, and with a capacity of 400 ml. Light was excluded by covers of thick brown paper, and the stoppers were split corks with a central hole for the insertion of the plant. A triangular wedge cut from the side of each cork provided additional aeration and a means of renewing liquid in the bottles. After 12 days, good root systems had developed, consisting of short, thick upper tap roots which thinned abruptly, fibrous roots at various levels, and, growing above the surface of the solution, horizontal feeder or surface roots.

There were three treatments each of eight plants. In the first, the lower part of the thick upper portion of the tap root was inoculated; in the second, two horizontal surface roots on each plant; in the third, a portion of the thin extension of the tap root well below the region inoculated in the first treatment. Inoculation consisted of rubbing a small area of root with the flattened end of a mounted needle dipped in the infective sap to which celite had been added. The roots were washed immediately after rubbing.

Sixteen days later, of the plants inoculated on upper and lower portions of the tap root, two in each group were infected in the tops, whereas of those inoculated on the surface roots, seven were infected in the tops.

In a similar test during winter, three groups each of fourteen plants were inoculated with tobacco mosaic, bushy stunt, and virus *X* respectively. Each group was halved, seven plants being inoculated at the base of the thick upper portion of the tap root, and seven on the surface roots. Leaves and roots of each plant were tested after 8 weeks. The results (Table 3) differed from those obtained with plants

growing in soil (cf. Table 1), as in this experiment bushy stunt caused fewest systemic infections.

TABLE 3. *Root infection of tomato plants growing in culture solution with tobacco mosaic, bushy stunt, and potato* X *viruses*

Virus	No. of plants inoculated	Site of inoculation	No. of plants infected in			
			Leaves	Roots	Root and leaves	Total no. infected
Tobacco mosaic	7	Tap root	0	4	2	6
Tobacco mosaic	7	Surface roots	0	1	5	6
Bushy stunt	7	Tap root	0	0	0	0
Bushy stunt	7	Surface roots	0	0	2	2
Virux *X*	7	Tap root	0	0	4	4
Virus *X*	7	Surface roots	0	1	4	5

Movement of virus in roots. A preliminary test was made with five young tomato plants growing in the glass containers. The roots were washed, and the fibrous roots separated into two approximately equal portions, one of which was tied as near the tap root as possible with waxed black thread. The roots below the thread were inoculated by rubbing between a finger and thumb moistened with a mixture of infected sap and celite. The roots were washed in running water, and the plants replaced in fresh culture solution. The whole of the top of the plant was then cut off immediately above the two seedling leaves. Tests of the inoculated and uninoculated portions of the roots a month later showed that none of the uninoculated portions was infected, but virus *X* was recovered from three of the five inoculated portions. The virus had evidently entered and multiplied locally but had not invaded the entire root system.

Two further tests with virus *X* included the following treatments:

(1) The fibrous roots were divided, one portion was marked as described above, and inoculated by rubbing with the fingers moistened with infective sap.

(2) The roots were treated as in (1), but after inoculation the top of the plant was cut off immediately above the two seedling leaves. Sprouts were removed from the stump as they developed.

(3) The thick upper portion of the tap root was marked half-way by tying with a thread. The portion below the marker was then inoculated by rubbing with a flattened needle dipped in the inoculum.

(4) As in (3), but after inoculation the roots were detached and suspended in flasks containing a little distilled water at the bottom. These were kept in the dark.

After inoculation each root was washed in running water to remove excess inoculum, but without allowing the stream of water to flow over the uninoculated portion. The date for testing the roots of the fourth treatment was determined by the condition of the detached roots; bacterial decay had started when the first samples were tested after 8 days, but the second samples were still relatively sound after 2 weeks.

When testing for the presence of virus, the inoculated and uninoculated portions of the roots were macerated separately. In treatments (3) and (4) the uninoculated portion comprised that part of the tap root, with its secondary roots, from just above the marker.

There was similar sunny weather during both tests, so temperature or light differences seem not to explain the fact that in the first test virus multiplied in eight and ten plants out of twelve in treatments (1) and (2), and in only two and four plants out of ten in the second. With treatment (3), in the first test none of the nine plants became infected, whereas in the second, of ten inoculated, virus was found in both the inoculated and uninoculated portions of the root in four, and had also moved to the leaves of three. Of twenty-one roots which were inoculated and then detached from the plants in the fourth treatment, virus had multiplied in one, where it was found in both the inoculated and uninoculated portions. White (1934), failed to infect roots of tomato plants growing in pure culture with tobacco mosaic virus either by direct inoculation or by contact with infected roots. The infection and multiplication of virus which occurred in the two tests described here may be due to physiological differences between roots inoculated and then immediately detached from a growing plant, and those which had grown continuously in culture solution.

TABLE 4. *Effect of site of inoculation with virus* X

Treatment	Date inoculated	No. of plants whose roots were infected in: Inoculated portion only	Uninoculated portion only	Both portions	No. of tops infected	No. infected out of no. treated
I. Half fibrous roots inoculated	21 July	8	0	0	0	8/12
	8 Sept.	1	0	1	0	2/10
II. As (I), but tops cut back to seedling leaves	21 July	8	0	2	.	10/12
	8 Sept.	3	0	1	.	4/10
III. Lower half tap root inoculated	21 July	0	0	0	0	0/9
	8 Sept.	0	0	4	3	4/10
IV. As (III), but tops detached and kept in dark.	21 July	0	0	1	.	1/9
	8 Sept.	0	0	0	.	0/12

The detached tops of the plants whose roots were used for the fourth treatment were utilized in two ways. Small drops of inoculum were placed on the cut surfaces of the nine plants used in the first test, after which the stems were replaced in bottles of culture solution. The plants regenerated root systems rapidly, and within 2–3 weeks most showed foliage symptoms of virus *X*. The roots were not tested. The twelve detached stems remaining from the second test had been cut so as to leave a few short adventitious roots developing at their bases, and these were inoculated by rubbing with a flattened needle dipped in inoculum. The plants were replaced in

culture solution and four became systemically infected; no virus was isolated from the roots of the others.

Effect of contact between roots of healthy and infected plants growing in culture solutions

Wide-mouthed glass bottles, capacity 650 ml., were used in a preliminary test with young tomato plants. The tops of the bottles were covered with waxed cardboard disks, each with two holes for the insertion of the plants, one of which was inoculated on the leaves with potato virus *X*. The other plant was covered with a cylinder of cellophane. These were not entirely satisfactory as growth of the plants tended to displace them, but to reduce the chances of contact between healthy and infected foliage, the inoculated plants were cut back to the seedling leaves as soon as systemic symptoms developed. Eight weeks after the start of the experiment, serological tests showed that four of the twelve uninoculated plants were infected with virus *X*.

Another experiment was made using the same containers but with a different type of screen between the healthy and infected plants. The containers were arranged in rows of six on the glasshouse bench, and cellophane screens, suspended from above and weighted along their lower edges, were hung so as to separate the pairs of plants in each bottle. Twelve pairs of tomato plants were grown in culture solution, and twelve in washed sand kept moist with culture solution. The experiment was started early in June, and 5 weeks later the leaves were tested serologically and the roots by inoculation to *Datura stramonium*. Six of the twelve uninoculated plants in culture solution contained virus in their roots only. Of the twelve uninoculated plants in the sand culture, one was infected in leaves and roots, and four others in the roots only. The provision of an abrasive medium for root growth did not increase the number of infections.

One test of the effect of contact between roots but not tops of diseased and healthy plants was made with potato plants, var. Majestic, in culture solution. The containers were large glazed pots of 2·5 l. capacity. Disks of strong cardboard impregnated with paraffin wax provided an upper covering, and similar disks, cut to fit exactly, were suspended inside the crocks about 1·5 in. from the top. This arrangement formed an upper chamber to support underground stems and developing tubers. Corresponding pairs of holes were made in the upper and lower covers, and, when the infected and healthy seed pieces were put in position their roots were inserted through the lower holes, giving access to the culture solution, and the shoots through the upper. The seed pieces had been previously sprouted in moist sand, and as much of the remaining tuber as possible was removed without damaging the roots or shoots. Twelve crocks were used, in six of which the upper chamber was subdivided with strips of cellulose acetate to prevent contact between underground stems and tubers. Screens of double cellophane separated the foliage of the healthy and diseased pairs of plants.

The experiment was started near the end of June, and tops, roots and tubers were

tested on *D. stramonium* plants 3 months later. Many of the tubers were hardly larger than peas, but each plant also produced some of 1–2 in. in diameter. These were tested by pooling portions from all the tubers from one plant as one sample. Five of the twelve originally healthy plants were found to be infected in the roots, four of which were from containers where underground stems, as well as the foliage, were also separated. None of the tubers tested at this time, or again in the following January, contained any virus.

Effect on plants of adding infective sap to culture solutions

It has already been shown that plants can be infected by adding infective sap to the soil in which they are growing, where possibly the abrasive action of the soil might aid root infection. Conditions to test whether completely undamaged roots can become infected are not easy to achieve. Roots of young tomato plants growing in culture solution in small containers are fragile, and even the minor disturbances necessary to replenish the liquid may cause injuries. In all experiments when infective sap was added to culture solution a proportion of the plants, usually about 10 %, became infected. Absence of any probability of root damage by movement of the containers was achieved only in two experiments, one of which was that described above with Majestic potatoes, and the other, described below, was when plants were grown in an apparatus in which culture solution circulated.

Tests were made to get information on whether a diseased plant can cause others to become infected without direct physical contact between them. If virus is released in some way from the roots, or washed into the soil after being leached from plant residues on the surface, such a medium might be a source of infection for the plants.

The form of apparatus used was varied slightly from time to time but the principle was the same throughout. A series of three or four containers, each of 2·5 l. capacity and with an outlet at the base, was arranged on wooden blocks on the glasshouse bench in descending levels. Each container was fitted with an inverted U-shaped glass inlet tube, one arm of which discharged about 1 in. from the bottom of the containers, and an L-shaped outlet tube. The end of the vertical arm of the L was 1·5 in. from the top of the container, and the horizontal arm left it through a rubber bung which sealed the outlet in the base. The end of this arm was connected by rubber tubing to the corresponding end of the inlet tube to the next container. A continuous flow of culture solution was maintained through the containers by the siphon thus formed, the overflow entering the outlet tube of one and being discharged near the bottom of the next through the inlet tube. During most of these experiments the solution was raised from the lowest level container back to the highest by means of a compressed air pump, but in the last tests of the series circulation was maintained simply by allowing the overflow to drain into a reserve tank, and then pouring this several times daily back into a reservoir tank. This was fitted with a stopcock which was opened only enough to allow the solution to drip slowly back into the first

container. Loss by transpiration and evaporation was made up daily with tap water except about once in 2 weeks, when fresh culture solution was added.

Five to eight young tomato seedlings were set in each container. All the plants in one were inoculated with potato virus *X*, and this was then isolated from the others by a cylindrical screen of cellulose acetate.

In the earlier tests glass containers were used, and during the first four, iron was supplied to the plants by spraying the foliage with a solution of ferric chloride. This resulted in poor and obviously iron-deficient plants, and in all the succeeding experiments earthenware crocks were used with iron added to the culture solution as ferric citrate. In these conditions the plants grew well and compared very favourably with others grown in soil; they required to be pruned heavily at least once during each experiment, especially during the summer.

TABLE 5. *Tests for transmission of virus* X *through circulating culture solutions*

Exp.	Type of container	Duration of exp.	No. of tomato plants tested	No. of plants infected in		Source of iron supply in culture solution
				Roots	Tops	
1	Glass	22 Dec.–2 Feb.	12	1	0	Ferric chloride
2	Glass	17 Feb.–8 Apr.	16	0	0	Ferric chloride
3	Glass	3 May–June 14	16	1	0	Ferric chloride
4	Glass	8 July–3 Aug.	12	1	0	Ferric chloride
5	Glass	22 Sept.–4 Nov.	6	0	0	Ferric citrate
6	Glass	11 Nov.–18 Jan.	12	0	0	Ferric citrate
7	Glass	16 Feb.–13 Apr.	12	0	0	Ferric citrate
8	Glazed	15 Apr.–16 June	12	0	0	Ferric citrate
9	Glazed	3 Mar.–18 May	18	0	0	Ferric citrate
10	Glazed	23 May–26 July	15	0	0	Ferric citrate
11	Glazed	1 July–8 Aug.	10	0	0	Ferric citrate
12	Glazed	28 Aug.–4 Oct.	15	0	0	Ferric citrate
		Total	156	3	0	

Description in text.

The results are summarized in Table 5. In the earlier ones positive reactions for virus *X* from root tests were obtained on three occasions. These occurred only during those tests when the plants were poor and chlorotic, when it was observed that small decayed portions of roots were being carried from one container to another. In subsequent tests, when conditions for plant growth were satisfactory, the root systems remained white and firm until they were tested, and no other infections with virus *X* were ever recorded. It is probable, therefore, that the infections in the earlier tests resulted from actual contact with diseased root fragments. Out of 100 plants tested in the latter series of experiments, none became infected, and it is thought improbable that virus *X* spreads from the roots of diseased plants to healthy ones without actual contact. In the last three tests the containers were filled nearly to the tops with gravel, but the solution circulated through this also did not produce any infection.

As a routine, the culture solution itself was tested at the end of each experiment, but it was never found to be infective. In two of the later tests, the roots of the

diseased plants and of healthy roots in one of the containers were injured by rubbing with celite. This did not cause any of the latter to become infected.

When the roots of the tomato plants in these experiments were tested by inoculation to tobacco they almost all gave lesions of tobacco necrosis. This occurred very rarely in tests of plants growing in individual containers, and it seems likely that the tobacco necrosis virus was being spread from the occasional natural infection which occurs in these plants by means of the circulating culture solution.

A different kind of test with virus *X* was made with this apparatus, which was set with fifteen healthy young tomato seedlings, by adding 100 ml. of infective sap to the circulating culture solution and rubbing the roots of the plants in one of the containers with celite. After 2 days, the apparatus was drained, flushed several times with water, and then refilled with fresh culture solution. The solution was tested on tobacco after the sap had been in circulation for 2 days and found to be infective. Roots and tops of the fifteen plants were tested on *D. stramonium* after a month, and one plant was systemically infected; this came from the container where the roots had been deliberately damaged. The culture solution was tested again at this time, but was not infective.

Discussion

This work has confirmed that infection of the whole or part of a plant through its roots can occur with tobacco mosaic, tomato bushy stunt and potato *X* viruses in tomato, and with virus *X* in potato. Similar results occur whether the viruses are directly inoculated to the roots or are added to the medium in which the roots are growing. With the latter, infection presumably occurs through injuries occasioned during root growth, but no unequivocal evidence was obtained showing that wounds are essential. The damaging of roots some time before they come into contact with the viruses did not greatly increase the likelihood of their becoming infected, and if infections occur only through wounds then it seems that contact is necessary between viruses and freshly made wounds.

The results help to explain some of the apparent contradictions and inconsistencies of previous work on infection through roots. Fulton (1941) suggested that these were explicable because, although roots are easily infected directly, the viruses take a long time to enter the stem and cause leaf symptoms. The results of the experiments described here suggest that an additional reason may be the extreme variability in the behaviour of plants where roots become infected. Viruses do sometimes remain confined to the roots for several weeks, and then enter the stems, but this is not always so even with the same virus. For example, in a test with bushy stunt virus started in January, infective sap was applied to the soil around the roots of twelve young tomato plants and six became infected, five in roots and tops within 3 weeks. The same type of test repeated in June resulted in six out of ten plants becoming infected, and with the virus confined to the roots of four. Similarly, a test with tobacco mosaic made in June gave ten out of fifteen plants infected, nine fully.

When repeated in the following September, again ten out of fifteen were infected, but in nine the virus was found in the roots only. Differences in age and susceptiblity of host plants may have determined the differences between the results at different times, but the factors that lead to full systemic infection through roots remain unknown. Perhaps the most puzzling aspect of the problem of root infection is the variation in individual plants used in one experiment; when established in the roots of one plant, the virus spreads rapidly to the foliage, yet it remains in the roots of others.

Potato plants whose roots were inoculated produced infected tubers but rarely had infected foliage. This is comparable with the situation in field experiments (Roberts, 1948) when contact between healthy and diseased plants more often led to infections in tubers than to infection in foliage. It now seems reasonable to assume that such field plants become infected by underground contact, rather than by leaf contact and the virus passing down to the tubers without spreading through the haulm. Fulton (1941) thought that although roots may become infected by viruses, it was questionable whether this was important in the occurrence of virus diseases in the field. He suggested that leaf symptoms would occur only relatively late in the life of annual plants, and so would be of minor importance. This is probably often true, but the severe reactions produced in some tomato plants infected with bushy stunt virus through their roots show that it is not always so. In the production of virus-free potato 'seed', the possibility that virus *X* may be spreading to tubers and not being detected by foliage tests, is obviously important, and there is need for supporting such tests by subsequent ones on tubers.

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OBSERVATIONS ON BLINDNESS IN OATS

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The relative importance in North Wales of various factors said to cause blindness in oats was investigated. It is concluded that a direct attack on the developing panicles by frit fly larvae and by adults and larvae of thrips was of little importance in causing the blindness encountered. Most of the blindness appeared to result from adverse physiological conditions probably operative during early growth. Varying varietal susceptibility to blindness was noted, and the effect on the subsequent panicles of an early insect attack on the vegetative parts of the plant is discussed.

One of the conditions responsible for loss of yield in oats is known as 'blindness' or 'blast'. These terms are commonly used to describe a condition in which some of the spikelets are sterile, although the term blind has also been used to denote sterile grain.

Blind spikelets are white or pale green in colour, and on dissection the floral parts are found to be much reduced or absent. They appear to range from minute white vestiges to pale green forms which approach the size of normal spikelets. The rachillae of blind spikelets are also affected, being white or pale green in colour and corresponding to the condition of the spikelets which they suspend. In nearly all cases examination of blind spikelets failed to reveal any trace of mechanical damage.

In the Ministry of Agriculture and Fisheries Monthly Reports on the Incidence of Plant Pests and Diseases, from 1923 onwards, frequent references to blindness occur. In North Wales all oat crops appear to be affected to some extent by this condition.

Some investigators, notably Hewitt (1914), have attributed blindness to thrips activity in the developing panicles. Working in Canada he concluded that the cause was damage to the developing spikelets by *Anaphothrips striatus* Osborne (now called *Pseudoarticulella obscurus* (Muller)). In Great Britain, Sharga (1933) considered that blind spikelets were caused by damage inflicted during development by *Limothrips cerealium* Halliday. Roebuck (1920) obtained evidence to associate blindness with the frit fly (*Oscinella frit* L.); he suggested that there were three broods of the fly on the oat crop, and that blindness was the result of damage caused by a proportion of the larvae of an 'intermediate' brood feeding on the developing panicles.

In contrast to the results mentioned above, other investigators have attributed blindness to adverse physiological conditions. Elliot (1925) concluded that a comparatively low rainfall during May led to an increase in the incidence of blindness. In Canada, Derick & Forsyth (1935) showed that light and water were important

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factors in causing blindness; light was most effective in reducing the incidence of blindness when applied 'normally' and in conjunction with excess moisture. Late seeding and late tillers tended to favour the incidence of blindness, and the critical period of growth for blindness development as shown by the influence of light and water, appeared to be approximately 6–8 weeks after sowing. The adverse operation of these factors during an earlier period of growth led to a reduction in the average number of spikelets per panicle. Rademacher (1936) concluded that although in certain circumstances blindness could be caused by infestation of the developing panicles by *Stenothrips graminum* Uzel and *Limothrips cerealium* Haliday, he considered that it was chiefly due to lack of water and nutriment and in a lesser degree to other causes such as inadequate aeration of the soil and cold, sunless weather. Derick & Hamilton (1939) demonstrated a highly significant negative correlation between figures for blindness infestation and yield, and indicated that the physiological factors contributing to blindness might also adversely affect the yield of the remaining fertile spikelets.

Varying varietal susceptibility to blindness was noted by Mackie (1928) and by Derick & Hamilton; Mackie, however, goes further and suggests that resistance to blindness is inherited as a single Mendelian factor, but the results of a statistical analysis of the data presented do not confirm this suggestion.

The present investigation was made mainly for the purpose of determining which factors are most important in causing blindness under field conditions in North Wales. It was done by means of (*a*) a crop survey, and (*b*) a manurial trial, and in addition observations were made on variety trials, on a crop badly attacked by the frit fly and on oats grown under insect-free conditions.

The crop survey

Eighteen crops grown at eleven widely separated localities were examined periodically during the 1947 growing season. The variety, sowing date, manurial and other treatments, were noted for each crop, soil samples were taken for chemical analysis, and the following observations were made on each crop.

(1) *Assessment of damage by the spring generation of the frit fly*

Two adjacent parallel 1 ft. lengths of drill were taken at each of twenty sites distributed at random in a zig-zag across one diagonal of the field and the proportion of damaged plants at each site noted. The counts were performed about 6 weeks after sowing.

(2) *Examination of the developing panicles*

When the panicles were judged to be within about 3 weeks of emergence from the leaf sheaths five tillers were taken from each of ten random sites distributed over a diagonal of the crop. They were examined for frit fly larvae and thrips; the position in the panicle of the thrips found was also recorded.

(3) *Assessment of blindness for each crop*

Panicles growing along a 1 ft. length of drill were cut from each of ten random sites distributed over each crop as previously described. The number of blind spikelets and the total number of spikelets were recorded for each panicle, the latter being divided roughly into thirds—lower, middle and upper—and the number of blind spikelets in each third recorded separately.

(4) *Examination of grain for frit fly damage*

Approximately 100 ripe heads were taken from random sites within each crop; the grain was rubbed out, repeatedly coned and quartered until about 100 grains

TABLE 1. *Showing numbers of frit fly, thrips and percentage blindness in a number of oat crops in North Wales*, 1947

	Locality	Variety	Sown	Frit fly: 1st generation, % plants damaged	Frit fly: 'Inter' generation, damage per 50 tillers	Frit fly: Grain generation, damaged grain (%)	Thrips No./50 developing panicles	Blindness (%)
1.	Denbighshire	S225	22 Apr.	0	3	3	3	22·6
2.	Denbighshire	S84	18 Apr.	0	0	2	2	34·6
3.	Flintshire	S84	19 Apr.	0	0	0	0	28·7
4.	Flintshire	Victory	22 Apr.	0	0	4	3	29·7
5.	Anglesey	Victory	28 May	0·70	5	0	7	30·7
6.	N. Caern.	Star	25 Apr.	0	2	0	15	20·5
7.	S.E. Caern.	Eagle	18 Apr.	0·30	0	0	0	28·3
8.	S.W. Caern.	Onward	5 May	0	3	0	0	21·0
9.	S.W. Caern.	S84	1 May	0·38	1	2	1	35·7
10.	S.W. Caern.	Star	14 May	2·7	—	0	—	30·6
11.	S.W. Caern.	S221	14 May	1·7	—	0	—	40·6
12.	S.W. Caern.	E. Miller	14 May	3·6	—	2	—	34·6
13.	S.W. Caern.	Spitfire	14 May	1·8	—	2	—	32·8
14.	S.W. Caern.	G. Rain	14 May	3·5	—	1	—	46·3
15.	S. Caern.	Eagle	12 May	0·23	—	0	—	14·1
16.	S. Caern.	Star	12 May	0·66	—	1	—	38·5
17.	S. Caern.	S221	12 May	0·13	—	0	—	22·7
18.	S. Caern.	S225	12 May	0·64	—	0	—	26·0

were left, and the percentage of grain damaged recorded. The results of this survey are summarized in Table 1. Notable points are as follows:

(*a*) The general high incidence of blindness. Blind spikelets were distributed within the panicles as follows: 85·3 % of those recorded occurred in the lower thirds of the panicles, 14·1 and 0·6 % occurring in the middle and upper thirds respectively.

(*b*) The general low incidence of damage by the spring and grain generations of the frit fly, despite the fact that many of the crops were late sown.

(*c*) The 'intermediate' generation of the frit fly. In one case (crop 5) the incidence of damaged tillers was high (10 %), but the damaged panicles had a characteristic

appearance, being malformed and twisted, and during the subsequent blindness counts only one such panicle occurred in the samples taken.

(*d*) The general incidence of thrips in the developing panicles was also low and the insects appeared to exhibit a preference for the spikelets in the upper parts of the panicles, 83·9 % of those recorded occurring in or around these spikelets.

The manurial trial

Whereas a crop survey was thought to be the best method of investigating the relationship between insect damage and blindness, a manurial trial was made in an attempt to assess the importance of nutritional conditions and variation in sowing date.

The trial occupied an area of a quarter acre on a field with the following cropping history: 1941, old pasture; 1942, oats and peas (undersown); 1943–4, ley receiving 5 cwt./acre basic slag; 1945, oats; 1946, oats.

The treatments consisted of all combinations of the following factors:

(*a*) Three sowing dates—(1) 14 April; (2) 28 April; (3) 12 May.

(*b*) No nitrogen v. 1½ cwt. sulphate of ammonia per acre (N).

(*c*) No phosphate v. 3 cwt. superphosphate per acre (P).

(*d*) No potash v. 2 cwt. sulphate of potash per acre (K).

There were six blocks each of four plots; the sowing date treatments were applied to whole blocks and the NPK and NPKS interactions were confounded with block differences. As there was only a single replicate the remaining three factor interactions with six degrees of freedom, were used as an estimate of error.

The variety of oats chosen for the trial was S. 84, a variety reputed locally to be particularly susceptible to blindness and especially so when grown on land of low fertility. The seed, previously treated with an organic mercury seed dressing, was sown at 2 cwt/acre; a heavy seed rate was used as the seed had to be sown broadcast and all cultivations except ploughing had to be done with hand implements; furthermore, a heavy seed rate is the usual farming practice for the district.

The observations made in the crop survey were repeated in each of the trial plots, assessment of damage by the spring generation of the frit fly being based on rectangular sampling units, other assessments being based on grab samples. Rectangular yield samples were also cut from each plot and the yield of grain determined.

The examination of the developing panicles was made during a more advanced stage of growth than in the crop survey, the panicles being within about 1–2 weeks of emergence from the leaf sheaths.

The effects of the treatments on spikelet number, blindness and yield, are shown in Tables 2 and 3. None of the differences between sowing dates was significant, but there was an indication that yield declined with later sowing. Spikelet number/panicle was increased by nitrogen, and there was a significant PK interaction; phosphate and potash both depressed spikelet number when applied alone, but caused a small increase when applied together. All fertilizer treatments tended to

reduce the percentage of blind spikelets, but only the nitrogen effect approached significance. Yield of grain was increased by the nitrogen and potash applications. There were no significant interactions between sowing date and fertilizers.

TABLE 2. *Effect of sowing date on spikelet number, blindness and yield of oats*

	Sowing date			
	14 Apr.	28 Apr.	12 May	S.E.*
No. of spikelets per panicle	23·8	32·2	27·8	2·75
Blind spikelets (%)	39·0	37·0	40·4	1·18
Yield of grain (lb./5 sq.yd.)	2·68	2·35	2·02	0·217

* 3 D.F.; $\sqrt{2}t = 4{\cdot}50$, $p = 0{\cdot}05$.

TABLE 3. *Effect of manures on spikelet number, blindness and yield of oats*

		Main effects			Interactions			
	Mean	N	P	K	NP	NK	PK	S.E.*
No. of spikelets per panicle	27·9	4·7	0·6	2·8	−0·0	1·3	4·4	1·43
Blind spikelets (%)	38·8	−3·4	−1·7	−1·4	−0·0	0·2	−0·6	1·43
Yield of grain (lb./5 sq.yd.)	2·35	0·56	0·06	0·46	0·10	0·22	0·11	0·144

* 6 D.F.; $t = 2{\cdot}45$, $p = 0{\cdot}05$

The results of observations on the incidence of insect damage can be summarized as follows:

(i) *Frit fly*

(*a*) *Spring generation*

An average of 0·30, 4·46 and 13·26 % damaged plants occurred in plots sown on dates 1, 2, and 3 respectively.

Manurial treatment induced no significant differences, but the increase due to delay in sowing date was highly significant (cf. Cunliffe, 1925).

(*b*) '*Intermediate*' *generation*

An average of 0·89, 3·22 and 3·19 % damaged tillers occurred in plots sown on dates 1, 2 and 3 respectively, the incidence of damaged tillers for date 1 being significantly lower than for dates 2 and 3. Manurial effects were not significant.

(ii) *Thrips*

Examination of the developing panicles revealed a thrips population per 100 panicles of 21·86, 95·34 and 37·54 in plots sown on dates 1, 2 and 3 respectively. The plots sown on date 2 had a significantly higher population than those sown on dates 1 and 3. Manurial effects were not significant. 78·3 % of the thrips were in or around spikelets on the upper thirds of the panicles.

The distribution within the panicles of the blind spikelets recorded in the trial was as follows: in the lower thirds of the panicles 82·8 %, in the middle and upper thirds 16·8 and 0·4 % respectively.

Other observations

(A) *Varietal susceptibility*

Blindness assessments were made on plots of variety trials of spring and winter oats in 1948. The spring oat varieties were S 84, Star, S 225 and Eagle, the same series being grown together at four centres and the winter varieties Picton, S 172, S 147, were grown together at three centres. No significant difference due to variety was established in the case of spring oats, but in the case of winter oats Picton exhibited a significantly lower incidence of blindness than either S 172 or S 147, the difference being due to variety.

The average incidence of blindness in the spring varieties was 25·9 % and in the winter varieties 27·7 %.

(B) *Oats badly infested by frit fly*

A very heavy infestation of frit fly occurred in a crop of Black Supreme oats sown 25 March 1948 at Chirk, Denbighshire, practically every plant showed symptoms of attack. The crop was kept under observation from early June onwards and the following observations were made:

(1) Stem attack can apparently persist throughout the growing season, especially in the late tillers, many of which are probably the result of the initial attack by the spring generation.

(2) The average incidence of blindness in the panicles formed by 30 July was 26·6 %, while the percentage of emerged panicles showing signs of mechanical damage by frit fly larvae was only 1·6.

(3) Many of the late tillers failed to form panicles, and those which eventually reached maturity appeared to form panicles which contained a small number of spikelets (fertile and infertile), i.e. they had a low spikelet number. The average spikelet number for the crop which takes into account the panicles formed by the earlier tillers was 13·1, whereas the same variety unattacked, but grown in a different locality in 1944 and sown on 20 April, had an average spikelet number of 34·7.

(C) *Oats grown under insect-free conditions*

In order to ascertain to what extent blindness could occur under insect-free conditions and to note the characteristics of any blind spikelets formed, oats (S 84) were grown in washed sand in an insect-proof glasshouse. Conditions in the house were generally unfavourable for growth, and the average incidence of blindness in the panicles formed was 51·3 %; 76·7 % of the blind spikelets occurring in the lower thirds of the panicles, 19·7 and 3·6 % occurring in the middle and upper thirds respectively. The blind spikelets were apparently similar to those formed under field conditions.

Species of Thysanoptera encountered

Larvae and adult females of *Stenothrips graminum* Uzel and *Limothrips cerealium* Hal. and small numbers of *Aptinothrips rufus* (Gmelin) were found in the developing panicles. In the mature heads of spring oats in August, large numbers of larvae of *Stenothrips graminum* were found and these were accompanied by adults of *Limothrips cerealium*. The ripe heads of winter oats in July were found to contain large numbers of larvae and some adults of *L. cerealium* but only very few individuals of *Stenothrips graminum*.

Pseudoarticulella obscurus (Muller) (= *Anaphothrips striatus* Osborne) was not found on oats in North Wales.

Discussion

Insect activity in the developing panicles as a cause of blindness

Frit fly

Roebuck (1920) considered that blindness was due to damage to the developing panicles by larvae of an 'intermediate' brood of the frit fly. Evidence from the present survey suggests that this is most unlikely. While in many of the crops examined the incidence of all generations of the frit fly was low, that of blindness was invariably high, and in the severely attacked crop at Chirk blindness was no higher than in many of the unattacked crops. Again, in crops where frit fly larvae were found in the developing panicles the damage inflicted was easily recognized and very few such damaged panicles were subsequently encountered in the emerged state. Even in the badly attacked crop at Chirk only 1·6 % of the panicles which were formed by 30 July showed signs of previous damage. Observations on this crop suggest that a late stem attack is commoner in late tillers, and it would seem that very few of the attacked tillers succeed in producing panicles.

It is concluded that a direct attack on the developing panicles by frit fly played little part in causing the blindness encountered. Cunliffe (1924) concludes that there are only two generations of the fly on the oat crop and that the larvae present in the developing panicles are either late larvae of the spring generation or early larvae of the generation normally attacking grain.

Thrips

Thrips activity in the developing panicles was considered to be the cause of subsequent blindness by Hewitt (1914), and by Sharga (1933), the former citing *Pseudoarticulella obscurus* (Muller), and the latter *Limothrips cerealium* Hal. as the species responsible.

Evidence from the present survey suggests that the blindness encountered was not caused by thrips activity.

The distribution of blind spikelets in affected panicles indicates that blindness is much commoner amongst spikelets in the lower parts of the panicle, whereas during

the examinations of developing panicles the majority of the thrips found occurred in the upper parts of the panicles.

In many crops the thrips population in the developing panicles was low, in crops 10–18, grown on exposed land at Pistyll and Aberdaron, Caernarvonshire, the developing panicles were not examined, but examination of the mature panicles in August revealed an extremely low thrips population—of the order of one or two individuals per panicle. On the other hand, although the population of thrips in mature panicles of crops grown in more sheltered areas was enormous, the incidence of blindness was high in all crops.

Pseudoarticulella obscurus was not found on oats in North Wales, the species most commonly encountered on spring oats being *Stenothrips graminum*, together with smaller numbers of *Limothrips cerealium*.

It is considered, therefore, that both frit fly and thrips activity in the developing panicles was of little importance in relation to the blindness condition found in North Wales.

Blindness as a result of adverse physiological conditions

Direct damage by insects having been considered to be unimportant the effect of physiological factors has to be considered. In this category possible effects on the panicles of an insect attack on parts of the plant remote from the actual panicles themselves may be involved.

The occurrence under insect-free conditions of blindness similar to that occurring under field conditions, and the absence of signs of mechanical damage suggest a physiological cause. Furthermore, the fact that many blind spikelets are in the form of white vestiges suggests that development has been arrested at an early stage. The distribution of blind spikelets within the panicle indicates that spikelets in the lower thirds are most susceptible to blindness. Bonnet (1937) has shown that the oat panicle is a many-branched determinate inflorescence, and the general application of this mode of development is that the first formed spikelets are those in the upper parts of the panicle and in the distal parts of the lateral branches. Thus the later-formed spikelets are found in the lower parts of the panicle, and accordingly it would seem that blindness is more prevalent amongst the later-formed spikelets. An explanation for such a distribution might be that blindness is partly due to an inability on the part of the plant to nourish adequately all the spikelet primordia formed. If so the later-formed spikelets would suffer most.

The results of the manurial trial suggest that potash is of little importance in relation to blindness, for the initial potash status of the soil was generally low and its influence on grain yield was significant. Nitrogen induced a significant increase in the average spikelet number per panicle (fertile and infertile spikelets) but the reduction in the incidence of blindness observed in plots treated with nitrogen was barely significant.

In contrast to Derick & Hamilton's (1939) results, delay in sowing did not induce

a significant effect on the incidence of blindness; but since only 3 days elapsed between ploughing and sowing the first series of plots, the short period of weathering probably counteracted the possible beneficial effect of early sowing.

From a consideration of the results of the present survey and those of Derick and his colleagues it would seem that the majority of the blindness encountered arose as a result of adverse physiological conditions probably operating during early growth.

There remains to be considered the effect of an insect attack on parts of the plant other than the panicles themselves. In the case of frit fly, observations on the badly 'fritted' field indicated that while the incidence of blindness was high, the main effect on the subsequent panicles was an apparent reduction in the total number of spikelets formed. Cunliffe *et al.* (1925) has shown that oats are most susceptible to an attack by the first generation of the fly when in the 2–3-leaved stage, i.e. about 3–4 weeks after sowing, and this period of growth corresponds to the critical period for spikelet formation as shown by the influence of light and water (Derick & Forsyth). It would seem, therefore, that the main attack by the first generation of the fly takes place before the critical period for blindness development (6–8 weeks after sowing). On the other hand, one of the effects of an early frit fly attack is the formation of a large number of late tillers which have been shown by Derick to be more susceptible to blindness.

It is probable, therefore, that an early attack of frit fly has a considerable effect on the subsequent panicles influencing both spikelet number and blindness, although the main effect appears to be a reduction in the spikelet number.

With regard to thrips, large populations of these insects do not occur on the plants until late in the growing season, and for this reason they are probably unimportant in relation to blindness. Large numbers of such sap-feeding insects, however, are nearly bound to effect the final yield, and probably do so by adversely affecting the maximum development of grain in the remaining fertile spikelets.

A note on blindness and fungus disease

It is well known that blindness in the form of whiteheads or take-all of oats arises as a result of an attack of *Ophiobolus graminis* Sacc., and particularly *O. graminis* var. *avenae* Turner (Turner, 1940). In barley, Millard & Burgess (1927) showed that blindness could be caused by *Helminthosporium gramineum* Rabenh., both by a direct infestation of the ears, and indirectly in the form of leaf stripe.

In the present survey, the relationship between blindness and fungus disease was not closely investigated but no severe fungus infestation was encountered. Whether a mild and less obvious infestation can be a contributory cause is not known.

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OBSERVATIONS ON THE BEET EELWORM AND OTHER CYST-FORMING SPECIES OF *HETERODERA*

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(With 8 Text-figures)

An account is given of observations made between 1944 and 1948 on beet eelworm and related species of *Heterodera*. The host ranges of beet-, potato-, pea-, carrot- and *Galeopsis*-root eelworms were studied in the field, on sites where previous information had been obtained of the species of *Heterodera* present. The beet eelworm attacked plants in the Polygonaceae, Chenopodiaceae, Amarantaceae, Aizoaceae, Caryophyllaceae, Cruciferae, Onagraceae and Labiatae. Considerable differences were observed in the intensity of attack upon different hosts. The Cruciferae contains many efficient hosts and is the most important host family in the British flora. The *Galeopsis* eelworm, like the beet eelworm, attacked plants in several families, including the Chenopodiaceae, Caryophyllaceae and Labiatae, which also contain hosts of the beet eelworm. The other root eelworms were more specific, the carrot eelworm attacked only cultivated and wild carrots (*Daucus carota* L.), the potato eelworm attacked only plants in the genera *Solanum* and *Lycopersicum* (potato, *Solanum dulcamara* L. and tomato), while the pea eelworm attacked only peas (*Pisum*), beans and tares (*Vicia*). The potato eelworm did not attack *Solanum nigrum* L. or *S. sarrachoides* Sendt. and there was no evidence that potato eelworm is capable of attacking weeds in natural orders other than the Solanaceae. In pot-tests, the hop eelworm attacked only hops and nettles and the cabbage eelworm only plants in the family Cruciferae. The rates of development of cysts of the beet, potato, pea and carrot eelworms were studied in the field. In all four species, the rate of development was similar and varied with the soil temperature, being slow in spring, more rapid in summer, falling off during the autumn and becoming negligible during the winter. In the pea eelworm, drought caused delay in the invasion of rootlets by eelworm larvae and reduced the intensity of attack. Assuming adequate moisture, the maximum number of generations for all four species is between two and three per year. In practice, the number of generations is also governed by the vegetative period of the host crop. During the host-range trials and observations on rates of development, fresh cysts were examined in large numbers. Some differences in shape were observed between the larger cysts of the various species. There were also differences in colour and in the number of eggs extruded into the gelatinous egg sac. Analysis of length and breadth measurements suggested that size ranges and regression coefficients are somewhat more valuable than average lengths and breadths, and length-breadth ratios, which are usually given. The carrot-root eelworm (*Heterodera carotae* Jones) is described, and the status of the *Galeopsis*-root eelworm is discussed. The latter is distinct from beet eelworm and appears to be identical with a form found by Goffart in 1936, for which the name *Heterodera galeopsidis* is proposed. The observations here recorded do not support the theory of adaptability of root eelworms to new hosts.

Introduction

Between 1944 and 1948, the host ranges and rates of development of the beet eelworm (*Heterodera schachtii* Schm.) and certain other cyst-forming species of

Heterodera were studied. One of these species, the carrot-root eelworm (*H. carotae* Jones, 1950), is described; another, attacking hemp nettles (*Galeopsis* spp.) and other plants, is distinct from the beet eelworm and is probably the same as a form observed on *G. tetrahit* L. in Germany by Goffart in 1936. Throughout these studies, incidental observations were made on cyst shapes, cyst size ranges, and on the production of the gelatinous egg sac and the extrusion of eggs into it.

Host ranges

In any study of the host ranges of the cyst-forming species of *Heterodera* in the field, difficulties arise from the possibility that the infected soil may contain cysts of species other than the one under test. The fundamental approach to this subject would be to test plants in pots of sterile soil, sand or other suitable medium to which cysts of the appropriate species had been added. Objections to this procedure are the unnatural conditions presented to both plants and nematodes, and the labour necessary to obtain adequate replication. In the field, the danger from unwanted species of *Heterodera* can be largely overcome if trial sites are on land which has been under observation in preceding years and some knowledge of the species present has been gained. These difficulties do not apply with potato-root eelworm which has distinctive cysts.

Host range trials were made in fields, or with soil from fields, about which information had been gathered over several years. Where the trial was made in the field itself, various seeds collected or obtained from the Botanic Gardens at Kew and Cambridge were sown in rows from 3 to 5 yd. long and 1 ft. apart. In addition, a portion of the trial area was left uncultivated to produce a growth of the naturally occurring weeds. Wherever supplies allowed, the seed was sown thickly and left unsingled. The rows were immediately watered and lightly dusted with 5 % D.D.T. The stands obtained varied from a few plants to hundreds per row but, generally, they provided well above twenty plants for examination. A single well-grown plant with an adequate root system was considered satisfactory as a test of infection. A few rows occurred in each trial where the plants, although sufficient in number, were poorly developed and even when taken together, provided inadequate root area for examination. These were rejected and counted amongst the failures. Samples of susceptible plants were lifted from time to time to observe the progress of cyst development and, when this was regarded as sufficiently advanced, the whole trial was examined row by row.

For pot-tests, the soil was obtained from the appropriate field in clean sacks and, after mixing, was placed in new pots of 5 in. diameter. These were sown with seeds and sunk in fresh horticultural peat to prevent drying out during hot weather. After germination, the seedlings were thinned and watered regularly. As with the field trials, pots of susceptible plants were turned out from time to time to indicate the progress of cyst development and all pots were examined when development was judged to be sufficiently advanced.

The criterion of susceptibility adopted was the essentially practical one, namely, the presence of fully developed cysts upon the roots. Examination of the roots was made under a hand lens. In all cases root systems were checked to make certain that the infected rootlets were attached to the actual plant under examination and were not stray roots from neighbouring rows. In doubtful cases, where mature cysts were few, the attachment of the cyst was verified to make sure that there was no chance adherence and, in addition, a sample of roots was washed and stained by the lactophenol-acid fuchsin method (Goodey, 1937) to demonstrate the presence of intermediate stages within the roots.

The results from the main trials are listed in Table 1. Here, the families are arranged according to Engler's system (Willis 1931). Under families, crop plants appear first in alphabetical order. Other plants follow, also in alphabetical order, the latin names of the indigenous species being taken from Clapham (1946) and their common names from Bentham & Hooker (1930). The results of the few pot tests made with hop and cabbage root eelworms are given separately in Table 2.

TABLE 1. *Plants tested in field trials of the host ranges of* Heterodera *spp.*

(– = negative result; + = positive result; blank = no test; * = pot test; ? = doubtful, small cysts only.)

Plants	Common names	Beet eel-worm	Potato eel-worm	Pea eel-worm	Carrot eel-worm	*Galeopsis* eel-worm
GRAMINEAE						
Triticum vulgare Vill.	Wheat	–	–	–	–	–
Hordeum sativum Pers.	Barley	–	–	–	–	–
Avena sativa L.	Oat	–	–	–	–	–
Secale cereale Bieb.	Rye	–	–		–	–
Zea mays L.	Maize	–	–	–	–	–
Agropyron sp.	—	–				
Arrhenatherum elatius (L.) J. & C. Presl.	False oat-grass	–	–	–		–
Bromus mollis L.	Soft brome-grass	–				
B. secalinus L.	—	–				
Cynosurus cristatus L.	Crested dog's-tail	–	–	–		–
Dactylis glomerata L.	Cock's-foot	–	–			–
Festuca pratensis Huds.	Meadow fescue	–	–	–		–
Lolium multiflorum Lam.	Italian rye-grass	–	–	–		–
L. perenne L.	Perennial rye-grass	–	–	–		–
Phleum pratense L.	Timothy grass	–	–	–		–
Poa annua L.	Annual meadow-grass	–			*–	
P. pratensis agg.	Smooth stalked meadow-grass	–	–			
AMARYLLIDACEAE						
Allium cepa L.	Onion	–	–	–	–	–
A. porrum L.	Leek	–	–	–	–	–
LILIACEAE						
Asparagus officinalis L.	Asparagus	–			–	
Bulbine annua Willd.	—	–		–		
URTICACEAE						
Urtica dioica L.	Common nettle	–			*–	–
U. pilulifera L.	Roman nettle	–		–	*–	–
U. urens L.	Small nettle	–	–	–	–	–

Plants	Common names	Beet eel-worm	Potato eel-worm	Pea eel-worm	Carrot eel-worm	*Galeopsis* eel-worm
CANNABINACEAE						
Humulus lupulus L.	Wild hop	−				
POLYGONACEAE						
Emex spinosa Campd.	—	−				
Fagopyrum sagittatum Gilib.	Buckwheat	−	−	−	−	−
F. tartaricum Gaertn.	—	−				
Rheum rhaponticum L.	Rhubarb	*+				
Rumex crispus L.	Curled dock	*+		−		?
R. obtusifolius L.	Broad-leaved dock	+	−	−	−	−
Polygonum amphibium L.	Amphibious polygonum	*−	−			−
P. aviculare agg.	Knotgrass	−	−	−	−	−
P. convolvulus L.	Black bindweed	−	−	−	−	−
P. persicaria L.	Persicaria	+	−	−	−	−
CHENOPODIACEAE						
Beta vulgaris L.	Sugar beet	+	−	−	−	−
B. vulgaris L.	Mangold	+	−	−	−	+
B. vulgaris L.	Red beet	+	−	−	−	+
B. vulgaris L.	Spinach beet	+	−	−		−
Spinacia glabra Mill.	Summer spinach	+	−	−	−	+
S. oleracea L.	Winter spinach	+	−		−	
Atriplex hortensis L.	Garden orache	+			*−	−
A. patula L.	Common orache	+	−	−	−	−
Beta maritima L.	Wild beet	+	−	−	−	−
Chenopodium album L.	Fat hen	+	−	−	−	−
C. bonus-henricus L.	Good King Henry	+				
C. capitatum (L.) Aschers	—	+				
C. hybridum L.	Sowbane	+	−	−	−	−
C. murale L.	Nettle-leaved goosefoot	+	−	−		−
C. polyspermum L.	Many-seeded goosefoot	+	−		−	
C. quinoa Willd.	—	−				
C. rubrum L.	Red goosefoot	+	−	−		
Chenopodium sp.	—	−				
C. vulvaria L.	Stinking goosefoot	+	−			−
Obione portulacoides (L.) Moq.	Sea purslane	*+				
AMARANTACEAE						
Amaranthus hypochondriacus L.	—	−	−	−		
A. quitensis H.B. & K.	—	+		−		
NYCTAGINACEAE						
Mirabilis jalapa L.	—	−				
PHYTOLACCACEAE						
Phytolacca acinosa Roxb.	—	−				−
AIZOACEAE						
Tetragonia expansa Murr.	New Zealand spinach	+	−	−		−
CARYOPHYLLACEAE						
Cerastium vulgatum L.	Mouse-ear chickweed			−	*−	
Dianthus sp.	Carnation	*+				
Melandrium album (Mill.) Garcke	White campion					−
M. dioicum (L.) Coss. & Germ.	Red campion	−			*−	

Plants	Common names	Beet eelworm	Potato eelworm	Pea eelworm	Carrot eelworm	*Galeopsis* eelworm
M. noctuiflorum (L.) Fr.	Night-flowering campion	−				
Saponaria officinalis L.	Soapwort	+	−	−		+
S. vaccaria L.	—	+	−	−	*−	+
Silene cucubalus Wibel.	Bladder campion	−	−	−	*−	−
S. pendula L.	—	−				
Spergula arvensis L.	Spurry	−	−	−	*−	−
Stellaria media (L.) Vill.	Chickweed	+	−	−	−	+
Tunica saxifraga Scop.	—	−				
Viscaria vulgaris Bernh.	Viscid campion	−				
RANUNCULACEAE						
Adonis annua L.	Pheasant's eye			−		
Delphinium sp.	Dwarf delphinium	−			−	
Nigella sp.	Love-in-a-mist	−		−	−	−
PAPAVERACEAE						
Argemone mexicana L.	Mexican poppy	−				
Escholtzia sp.	—	−				
Papaver rhoeas L.	Field poppy					−
CRUCIFERAE						
Brassica napus L.	Coleseed or rape	+	−		−	
B. napus L.	Swede	+	−	−		−
B. nigra (L.) Koch.	Black mustard	+				
B. oleracea L.	Broccoli	+				
B. oleracea L.	Brussel's sprout	+				
B. oleracea L.	Cabbage	+	−	−	−	
B. oleracea L.	Cauliflower	+				
B. oleracea L.	Kale	+				
B. oleracea L.	Kohl rabi	+				
B. rapa L.	Turnip	+	−	−	*−	−
Isatis tinctoria L.	Woad	+				
Lepidium sativum L.	Cress	+	−	−	−	−
Raphanus sativus L.	Radish	+	−	−	−	−
Sinapis alba L.	White mustard	+		−	−	−
Alyssum sp.	Alyssum	*−			*−	−
Armoracia lapathifolia Gilib.	Horseradish	+				
Aubretia sp.	Aubretia	+			*−	
Brassicella erucastrum (L.) O. & E. Schultz	—	+				
Capsella bursa-pastoris (L.) Medic.	Shepherd's purse	+	−	−	−	−
Cheiranthus allionii Hort.	Siberian wallflower	+	−			
C. cheirii L.	Wallflower	+	−	−	*−	−
Cochlearia officinalis L.	Scurvy grass	*+				
Coronopus squamatus (Forsk.) Aschers	Wart cress	?	−	−		
Descurainia sophia (L.) Prantl.	Flixweed	+			−	
Erysimum cheiranthoides L.	Treacle mustard	+	−	−	*−	−
Iberis sp.	Candytuft	+	−			
Lunaria annua L.	Honesty	+				
Matthiola sp.	Garden stock	−				−
Raphanus raphanistrum L.	Wild radish	+	−	−	*−	−
Sinapis arvensis L.	Charlock	+		−	*−	−

Plants	Common names	Beet eel-worm	Potato eel-worm	Pea eel-worm	Carrot eel-worm	*Galeopsis* eel-worm
CRUCIFERAE (*continued*)						
Sisymbrium officinale (L.) Scop.	Hedge mustard	+	–			
Thlaspi arvensis L.	Penny cress	+				
RESEDACEAE						
Reseda alba L.	White mignonette	–	–	–		–
ROSACEAE						
Agrimonia eupatoria L.	Agrimony	–				
Poterium sanguisorba L.	Salad burnet	–		–		–
LEGUMINOSAE						
Anthyllis vulneraria L.	Kidney vetch	–	–	–		
Lathyrus odoratus L.	Sweet pea	–	–	–		
Medicago lupulina L.	Black medick			–†		
M. sativa L.	Lucerne	–	–	–	–	–
Onobrychis viciifolia Scop.	Sanfoin	–	–	–	–	–
O. viciifolia Scop.	Giant sanfoin			–†		
Phaseolus multiflorus Willd.	Runner bean	–	–	–	–	?
P. vulgaris L.	French bean	–		–		?
Pisum sativum L.	Garden pea	–	–	+	–	–
Trifolium dubium Sibth.	Suckling clover			–†		
T. hybridum L.	Alsike			–†		
T. incarnatum L.	Crimson clover			–†		
T. pratense L.	Broad red clover	–	–	–	–	+
T. pratense L.	Single cut red clover			–†		
T. repens L.	Wild white clover	–	–	–	–	–
T. repens L.	Dutch white clover			–†		
Vicia faba L.	Field bean			+		
V. faba L.	Broad bean	–	–	+	–	–
V. sativa L.	Vetch			+		
V. sativa L.	Winter vetch	–	–	+	*–	–
GERANIACEAE						
Erodium mancescavi Coss.	—	–				
Geranium rotundifolium L.	Round-leaved crane's-bill	–		–		–
LINACEAE						
Linum anglicum Mill.	Perennial flax	–		–		–
Linum sp.	—	–	–		–	–
RUTACEAE						
Dictamnus albus L.	—	–				
EUPHORBIACEAE						
Euphorbia exigua L.	Dwarf spurge				–	
E. helioscopia L.	Sun spurge	–		–	–	–
E. lathyrus L.	Caper spurge	–				
MALVACEAE						
Malva sylvestris L.	Common mallow	–	–	–	*–	–
VIOLACEAE						
Viola tricolor agg.	Heartsease	–	–		*–	–
ONAGRACEAE						
Lopezia coronata Andr.	—	+	–	–		–
Oenothera biennis L.	Evening primrose	–	–			–
UMBELLIFERAE						
Apium graveolens L.	Celery	–	–	–	–	
A. graveolens L.	Celeriac	–			–	

†=records from a trial carried out on the same land by J. H. Stapley in 1940 (*in litt.*).

Plants	Common names	Beet eel-worm	Potato eel-worm	Pea eel-worm	Carrot eel-worm	*Galeopsis* eel-worm
Daucus carota L.	Carrot	–	–	–	+	–
D. carota L.	Wild carrot				+	
Pastinaca sativa L.	Parsnip	–	–	–	–	
Petroselinum crispum (Mill.) Nym.	Parsley	–	–	–	–	
Aegopodium podagraria L.	Bishop's weed				*–	
Aethusa cynapium L.	Fool's parsley	–	–			
Anethum graveolens L.	Dill-seed	–			–	
Bupleurum rotundifolium L.	Hare's-ear				–	
Carum carvi L.	Caraway				–	
Chaerophyllum temulum L.	Rough chervil				–	
Conium maculatum L.	Hemlock	–	–		*–	–
Coriandrum sativum L.	Coriander				–	
Eryngium bromeliaefolium Delar.	—				–	
Foeniculum capillaceum Gilib.	Fennel				–	
Meum athamanticum Jacq.	Spignel				–	
Scandix pecten-veneris L.	Shepherd's needle	–		–		
Torilis anthriscus (L.) C. C. Gmel.	Hedge parsley				–	
T. nodosa (L.) Gaertn.	Knotted caucalis				–	
PRIMULACEAE						
Anagallis arvensis L.	Common pimpernel	–	–	–	*–	–
CONVOLVULACEAE						
Calystegia sepium (L.) R.Br.	Larger bindweed	–				
Convolvulus arvensis L.	Lesser bindweed	–				–
C. tricolor L.	—			–		–
POLEMONIACEAE						
Gilia abrotanifolia Nutt.	—	–		–		–
HYDROPHYLLACEAE						
Phacelia tennuifolia Harv.	—	–				–
BORAGINACEAE						
Cynoglossum officinale L.	Hound's tongue	–				–
Myosotis arvensis (L.) Hill.	Field forget-me-not	–			–	
Myosotis sp.	—	–		–		–
LABIATAE						
Ajuga chamaepitys (L.) Schreb.	Ground pine	–				–
Galeopsis pyrenaica Bartl.	—	+	–	–	*–	+
G. speciosa Mill.	Large-flowered hemp nettle	+	–			+
G. tetrahit L.	Hemp nettle			–		+
Hyssopus officinalis L.	Hyssop	–				
Lamium album L.	White dead nettle	–				
L. purpureum L.	Red dead nettle	–	–	–	–	–
Mentha arvensis L.	Corn mint	–				–
Salvia officinalis L.	Garden sage	–	–	–		–
S. sclarea L.	—	–				
Scutellaria orientalis L.	Skullcap	–				
Stachys annua (L.) L.	Woundwort	–				

Plants	Common names	Beet eel-worm	Potato eel-worm	Pea eel-worm	Carrot eel-worm	*Galeopsis* eel-worm
SOLANACEAE						
Solanum tuberosum L.	Potato	–	+	–	–	
Lycopersicum esculentum Mill.	Tomato	–	+	–	*–	–
Atropa bella-donna L.	Deadly nightshade		–			
Capsicum sp.	—		–			
Datura stramonium L.	Thorn apple	–	–	–	*–	–
Hyoscyamus niger L.	Henbane	–	–	–	*–	–
Nicandra physaloides Gaertn.	—		–			
Nicotiana langsdorffii Schrank	—		–			
N. paniculata L.	—		–			
N. sylvestris Speg. and Com.	—				*–	
N. tabacum	Tobacco		–			
Schizanthus pinnatus Ruiz. and Pav.	—		–			
Solanum dulcamara L.	Woody nightshade		+			
S. nigrum L.	Black nightshade	–	–	–	–	
S. sarrachoides Sendt.	—	–	–		*–	
SCROPHULARIACEAE						
Linaria sp.	Toadflax	–				–
Scrophularia nodosa L.	Figwort	–	–			
Veronica agrestis L.	Procumbent speedwell	–	–	–	–	+
PLANTAGINACEAE						
Plantago lagopus L.	—	–	–			–
P. major L.	Greater plantain	–		–	–	
RUBIACEAE						
Crucianella angustifolia L.	—	–	–			–
Galium aparine L.	Goose-grass	–	–	–	–	–
DIPSACACEAE						
Dipsacus lacineatus L.	Teasel	–	–	–		–
CUCURBITACEAE						
Cucurbita pepo L.	Marrow	–	–	–	–	–
COMPOSITAE						
Cichorium intybus L.	Chicory	–	–		–	–
Lactuca sativa L.	Lettuce	–	–	–		–
Achillea millefolium L.	Yarrow	–				
Arctium lappa L.	Burdock	–			–	
Artemisia vulgaris L.	Mugwort	–		–	–	
Bidens pilosa L.	—			–		–
Cirsium arvense (L.) Scop.	Common thistle	–			–	–
Gnapthalium uliginosum L.	Marsh cudweed		–			
Helianthus sp.	Sunflower				–	
Lapsana communis L.	Nipplewort		–			
Matricaria maritima L.	Scentless mayweed		–		–	–
Senecio vulgaris L.	Groundsel	–		–	–	–
Sonchus sp.	Sowthistle	–			–	
Totals		183	110	109	105	109

TABLE 2. *Results of pot-tests with hop- and cabbage-root eelworms*

Plants	Common names	Cruciferous eelworm	Hop eelworm
CRUCIFERAE			
Brassica napus L.	Coleseed	+	
B. napus L.	Swede	+	
B. oleracea L.	Kohl rabi	+	
B. rapa (L.) Koch.	Turnip	+	
Biscutella didyma L.	—	−	
Cheiranthus cheirii L.	Wallflower	+	
Raphanus sativus L.	Radish	+	
Sinapis alba L.	White mustard	Failed	Failed
CANNABINACEAE			
Humulus lupulus L.	Wild hop	Failed	+
URTICACEAE			
Urtica dioicia L.	Common nettle	−	+
U. urens L.	Small nettle		+
OTHER FAMILIES			
Avena sativa L.	Oat	−	−
Beta vulgaris L.	Sugar beet	−	−
Daucus carota L.	Carrot	−	−
Galeopsis pyrenaica Bartl.	Hemp nettle	−	−
Myosotis sp.	Forget-me-not	−	−
Solanum tuberosum L.	Potato	−	−
Stellaria media (L.) Vill.	Chickweed	−	−
Trifolium pratense L.	Red clover	−	−
Veronica agrestis L.	Procumbent speedwell	−	−
Pisum sativum L.	Pea	Failed	Failed

Beet eelworm

The first host range trial of beet eelworm (*H. schachtii* Schm.) was made in 1945, in a fenland field which had been under observation since 1935 and used for the investigation of soil populations (Jones, 1945, field 17). Apart from a single cyst found on oats during attempts to estimate the susceptibility of certain host crops (Petherbridge & Jones, 1944, Table 1), no evidence of the presence of other species of *Heterodera* had been obtained, and the land was considered to contain an almost pure culture of beet eelworm. The last susceptible crop grown was cress seed in 1935. By 1945 the eelworm population had fallen considerably, but test plots sown in 1943 and 1944 showed that it was sufficient to give a heavy and uniform infection of sugar beet without causing 'beet-sickness'. In 1945, the seeds of 125 different plants were sown and twenty-eight rows failed or were discarded. Seeds of thirteen varieties of commercial beet and forty-five 'families' of *Beta maritima* L. were also sown in the same trial.

The second trial was made in 1946, also in a fenland field which had been under observation since 1935 and used for the investigation of eelworm populations. Until the year of the trial, there was no evidence of the occurrence of other species of *Heterodera*. In that year, however, a very light infection of potato eelworm was observed near the trial area. The previous susceptible crop was sugar beet in 1940,

and the trial was in a portion of the field which had been 'beet-sick'. Test plots of beet sown in 1943 and 1944 showed that a heavy and uniform attack could be anticipated. In both trial fields, examination of crops and naturally occurring weeds had been made from time to time. In 1944, a re-check of the weeds was made as a preliminary to the trials. In the second trial, the seeds of 178 different plants were sown and fifty-seven rows failed or were discarded.

In addition to the field trials, twelve more plants were tested in pots using soil from the site of the first trial.

The results from the two field trials, which had nearly 100 different plants in common, were nearly identical. In the first trial, the previous susceptible crop was cress seed grown some 10 years previously so that the effective eelworm population had been largely produced on this host plant. Similarly, in the second trial, the effective host plant was sugar beet grown 6 and 8 years previously in 1938 and 1940. The results are also in agreement with extensive field observations (Petherbridge & Jones, 1944), much the same host range amongst common weeds having been observed in the fens, and at isolated sewage farms where the eelworm population must have been largely produced on mangolds.

As a further test of the homogeneity of the eelworm population on the 1945 trial ground, mature white cysts were collected from twenty-nine different susceptible plants and placed in separate pots of sterilized soil. These were then sown with beet and turned out after a suitable interval. In every pot, except that containing cysts from fat hen (*Chenopodium album* L.), the sugar-beet plant was found to be infected. The pots were again sown with beet in the spring of the following year and once more all beet plants were infected, with the exception on this occasion of the one containing cysts from soapwort (*Saponaria vaccaria* L.). Thus, successful transferences to sugar beet were obtained from all twenty-nine plants, the names of which are listed below in Table 3.

Amongst the plants found infected with beet eelworm, there appeared to be considerable variation in the degree of susceptibility as judged by the abundance of cysts upon the roots. In the family Chenopodiaceae, the cultivated varieties of *Beta vulgaris* L., sugar beet, red beet, mangold and spinach beet, were all heavily infected and produced an abundance of cysts. There was no obvious difference in the susceptibility of the thirteen commercial strains of sugar beet sown in the 1945 trials. The general appearance of the roots was so uniform that attempts to count cysts and to allot marks to the different strains were abandoned. Cysts were also formed freely by plants in all of the forty-five 'families' of *Beta maritima* tested in the same trial. A few individual roots of the rat-tailed type bore fewer cysts than average but not a single plant out of the 2000 plants examined was entirely free from cysts. In the genus *Chenopodium*, *C. capitatum* (L.) Aschers, *C. murale* L., *C. rubrum* L. and *C. vulvaria* L. formed cysts freely. Cysts were less abundant upon *C. bonus-henricus* L., *C. hybridum* L. and *C. polyspermum* L., while the very common weed *C. album* L. was very resistant, one or two cysts being found on about one plant out of very ten

TABLE 3. *Plants from which successful transferences of beet eelworm were made to sugar beet*

Plant	Common name
POLYGONACEAE	
Rumex crispus L.	Curled dock
R. obtusifolius L.	Broad-leaved dock
Polygonum persicaria L.	Persicaria
CHENOPODIACEAE	
Beta vulgaris L.	Sugar beet
B. vulgaris	Mangold
B. vulgaris	Red beet
Spinacia glabra Mill.	Summer spinach
S. oleracea L.	Winter spinach
Chenopodium album L.	Fat hen
C. bonus-henricus L.	Good King Henry
C. capitatum (L.) Aschers	—
C. polyspermum L.	Many-seeded goosefoot
C. rubrum L.	Red goosefoot
CARYOPHYLLACEAE	
Saponaria officinalis L.	Soapwort
S. vaccaria L.	—
Stellaria media (L.) Vill.	Chickweed
CRUCIFERAE	
Brassica napus L.	Swede
B. oleracea L.	Cabbage
B. rapa L.	Turnip
Isatis tinctoria L.	Woad
Lepidium sativum L.	Cress
Raphanus sativus L.	Radish
Sinapis alba L.	White mustard
Capsella bursa-pastoris (L.) Medic	Shepherd's purse
Cheiranthus allionii Hort.	Siberian wallflower
C. cheirii L.	Wallflower
Iberis sp.	Candytuft
Lunaria annua L.	Honesty
LABIATAE	
Galeopsis pyrenaica Bartl.	Hemp nettle

lifted. *C. quinoa* Willd. and another undetermined species of *Chenopodium* from South America produced no cysts at all. The two species of *Spinacia* and of *Atriplex* were somewhat less efficient hosts of beet eelworm than the varieties of *Beta vulgaris* L. In potted soil, a single plant of *Obione portulacoides* (L.) Moq. bore a few cysts.

In the Polygonaceae, the two common species of dock, *Rumex crispus* L. and *R. obtusifolius* L., produced cysts freely, whereas the weed *Polygonum persicaria* L. carried only an occasional cyst. Buckwheat, *Fagopyrum sagittatum* Gilib., and the other species of *Fagopyrum* tested appeared to be immune.

In the Caryophyllaceae, the soapworts, *Saponaria officinalis* L. and *S. vaccaria* L., were highly susceptible and produced an abundance of cysts. Only a few cysts were found on carnation cuttings grown in potted soil. The status of chickweed, *Stellaria media* (L.) Vill., was difficult to assess because it is an ephemeral. Although quite susceptible, an abundance of mature cysts is rarely produced. Mature cysts can, however, be obtained from the older rootlets of undisturbed plants.

In the Cruciferae, there were many efficient hosts. On species of *Sinapis*, it was sometimes difficult to find cysts on mature plants but the difficulty was partly due to the form of the root system. No cysts were found on *Matthiola* and *Alyssum* but these require further testing. In the roots of *Coronopus squamatus* (Forsk.) Aschers, swollen, third-stage larvae could be demonstrated by staining, but repeated attempts to find cysts met with failure. In this plant, larvae seem incapable of developing beyond the third stage.

Amaranthus quitensis H.B. & K. (Amarantaceae), *Tetragonia expansa* Murr. (Aizoaceae) and *Lopezia coronata* Andr. (Onagraceae) all produced cysts in moderate numbers. Only a few cysts were found upon *Galeopsis pyrenaica* Bartl. (Labiatae), and still fewer upon *G. speciosa* Mill., a common weed, which has much the same status in regard to beet eelworm as *Chenopodium album* L. and *Polygonum persicaria* L.

Some of the susceptible plants fall into families which are interrelated botanically. Thus, the Chenopodiaceae, Amarantaceae, Aizoaceae and Caryophyllaceae fall in Engler's cohort Centrospermae to which the Polygonaceae of the cohort Polygonales is allied. Other susceptible plants lie in isolated families without clear botanical relationships. The Cruciferae contains the greatest number of highly susceptible plants and is the most important host family in the British flora. It is not possible to say whether this statement is more generally true, since the 183 plants tested against beet eelworm represent only a tithe of possible host species.

Potato-root eelworm

The first host range trial of potato-root eelworm (*Heterodera rostochiensis* Woll.) was made in 1945, in a field which had been under observation since 1940. Extensive 'sick' patches appeared in the potato crop in 1942. The trial was located in one of these and examination of self-sown potatoes in 1944 indicated that a heavy attack could be anticipated. Observations on crops and weeds failed to reveal the presence of other species of *Heterodera* except for a very light infection with the cereal root eelworm (*H. major* O. Schm.). In this trial, the seeds of fifty-four plants were sown of which four failed or were discarded.

The second trial was undertaken in 1946 on a field where potatoes had suffered severely from eelworm attack in the preceding year. The soil was acid in reaction and the trial plot was therefore limed. No crops susceptible to beet eelworm had been grown since 1939. Examination of weeds failed to reveal the presence of other species of *Heterodera*. In the trial area, additional rows of potatoes were sown and these, together with volunteer plants, showed that the level of infection was reasonably uniform but not very heavy. The seeds of 103 plants were sown, of these twenty-nine failed or were discarded. No pot tests were made.

In the two field trials of the host range of potato-root eelworm, which had some fifty plants in common, cysts were found only upon the roots of potatoes, tomatoes and woody nightshade. These results are in general agreement with those of

Franklin (1940), while the immunity of common crops and weeds is supported by extensive field observations. A number of reports have been received from farmers and district officers of the occurrence of potato eelworm cysts upon the roots of sugar beet and common weeds but, where these have been investigated, it has been found impossible to substantiate them. In 1944, on the field in which the first potato eelworm trial was made, more than fifty sugar-beet plants were carefully examined for the presence of potato eelworm cysts without success, yet each plant was adjacent to a volunteer potato plant on the roots of which there was an abundance of cysts. In the trials, and in the field generally, no cysts of the potato eelworm were found on the roots of the common weed *Solanum nigrum* L. Nor could cysts be found upon *S. sarrachoides* Sendt., a foreign weed introduced in imported carrot seed and reported susceptible by Bates (1945). Samples of this plant were returned to the laboratory but no larvae could be found in the roots.

Pea-root eelworm

The pea-root eelworm (*Heterodera göttingiana* Lieb.) host range trial was made on heavy land near Cambridge where, in 1939, a crop of peas had suffered partial failure owing to eelworm attack. In 1945, preliminary observations were made upon the naturally occurring weeds. The trial area was also sown with peas to increase the eelworm population, a uniform and moderately heavy attack being observed. No evidence of the existence of other species of *Heterodera* could be found. In 1946, the seeds of 156 plants were sown, of which fifty failed or were discarded.

In the pea-root eelworm trial, the only plants found infected out of the 109 tested were pea, broad bean, field bean and tares. In three outbreaks investigated in the eastern counties, it was found that peas were devastated on land where it was alleged that no known host plant had been grown during recent years. This suggested extreme persistence of the pea eelworm, high susceptibility of the pea plant or the existence of unknown hosts amongst common crop plants and weeds. No evidence for the occurrence of additional hosts was found in the field trial or in field observations.

Carrot-root eelworm

The first trial of the host range of carrot-root eelworm (*Heterodera carotae* Jones) was made in a fenland field in the autumn of 1944, immediately after the lifting of an infected carrot crop. Strips of beet grown on different parts of the same field had been examined from time to time since 1939 without revealing the presence of beet eelworm, although this was present in the neighbourhood and on part of the same farm. Potato eelworm was present on potatoes in another part of the same field but appeared to be absent from the trial area. In the 1944 trial, thirty-six crop and garden plants were tested with eighteen species of naturally occurring weed. There were no failures or discards.

The second trial was made in the same field in the spring of 1945. The seeds of eighty-four different plants were sown and twenty-eight failed or were discarded.

In 1946, using soil from the trial area, forty-six pot tests were made of which nine failed.

The carrot-root eelworm, like the potato- and pea-root eelworms, was highly specific. The only plants attacked out of a total of 105 tested were cultivated and wild carrot. No evidence of larval invasion was found in the rootlets of the other Umbelliferae grown.

Galeopsis-*root eelworm*

The field chosen for the *Galeopsis*-root eelworm (*Heterodera galeopsidis* Goff.) trial was a fenland field which had been under observation since 1939. In that year, part of the field grew beet which suffered a moderate attack from beet eelworm. In 1941, the entire field was sown with beet, and examination showed that the beet eelworm was localized at the southern end of the field which had grown frequent susceptible crops. In the remainder of the field, a second species of *Heterodera* was found producing numerous distinctive yellow cysts upon certain weeds while, at that time, no cysts would be found on adjoining sugar-beet plants. In 1945, weeds were again examined and a site chosen for a trial in the following year. The naturally occurring weeds that grew on the trial area were left here and there to provide a guide to the uniformity and intensity of the attack.

The results of the *Galeopsis*-root eelworm trial were somewhat less satisfactory than those of the other species of *Heterodera* owing to the rather low level of infection over the trial area. Nevertheless, it was clear that the following plants were highly susceptible:

Caryophyllaceae	
Saponaria officinalis L.	Soapwort
S. vaccaria L.	Soapwort
Stellaria media (L.) Vill.	Chickweed
Labiatae	
Galeopsis speciosa Mill.	Hemp nettle
G. tetrahit L.	Hemp nettle
G. pyrenaica Bartl.	Hemp nettle
Scrophulariaceae	
Veronica agrestis L.	Procumbent speedwell

The species of *Galeopsis* in the above list were much more susceptible to *Galeopsis* eelworm than to beet eelworm. In the trial, seven out of every ten of the above plants were infected. By comparison, the varieties of *Beta vulgaris* L. showed considerable immunity, only four obvious cysts being noted upon eighty plants. Similarly, there were only two obvious cysts upon thirty plants of summer spinach. Cysts were not very numerous on red clover and none was observed on white clover. A few immature cysts were found upon runner beans and french beans and also upon a single plant of curled dock. No beet eelworm cysts were observed on any plant in the trial.

The host range of the *Galeopsis* eelworm is quite wide and may well be wider than indicated by this trial with its low level of infection. Overlap with the host

range of beet eelworm occurs in at least four families, but there is less evidence of botanical affinity between the families affected. Host plants of both the pea and *Galeopsis* eelworms occur in the Leguminosae, but, so far as is known, the same plants do not serve as hosts for both species of *Heterodera*.

Hop- and cabbage-root eelworms

Supplies of soil containing hop root eelworm (*Heterodera humuli* Filipjev) were obtained from a hop garden in Kent and of cabbage-root eelworm (*H. cruciferae* Franklin) from a field in the Holland Division of Lincolnshire. The latter also carried a moderate infection of potato-root eelworm. In both cases, a limited number of pot tests were made with the infected soil. The cabbage-root eelworm attacked only Cruciferae and the hop-root eelworm only hop and nettles, the latter being plants in closely related families (Cannabinaceae and Urticaceae). The potato, pea, carrot, cabbage and hop eelworms show no overlap in host ranges, although the cabbage-root eelworm overlaps the beet eelworm in the family Cruciferae.

Rate of development of cysts in the field

Preliminary work

In 1944, preliminary work was done to find whether some simple linear measurement, such as cyst length or breadth, could be used in a study of the rate of development of beet-eelworm cysts in the field, or whether this might be conveniently replaced by observation of the various stages passed through during growth. Successive sowings of sugar beet, coleseed and white mustard were made throughout the summer months on the site of the first beet-eelworm host range trial. Each sowing was heavily watered to ensure that there was no delay in germination. Samples of plants were collected weekly from each plot throughout the growing season and, in the laboratory, white cysts were collected from the roots or, where cysts had not developed, the roots were stained with acid fuchsin in lactophenol to determine the stage reached by the larvae. Length and breadth measurements of the cysts were made under a low-power monocular microscope with the aid of a calibrated micrometer eyepiece. The cysts were then opened to ascertain the stage reached in egg production. Two difficulties were encountered at the outset. The first was due to the invasion of the seedling rootlets being spread over a considerable period, so that, on any sample of roots, there were cysts in a whole range of different sizes and stages. The second difficulty was that many cysts were arrested or retarded in growth by such causes as displacement from the host rootlet, or the death or malnutrition of the host rootlet. Thus, two small cysts of equal size often exhibited different stages of internal development, the older, arrested in growth, might contain embryonated eggs, while the younger, still growing, had not yet begun egg production. The first difficulty was overcome by following the development of cysts arising from the first larval invasions which take place immediately after germination. The cysts produced by

these larvae lie along the developing tap root or close to it along the first formed side roots. The second difficulty was avoided by discarding from these cysts all but the largest and most forward which had suffered no hindrance of growth. The relationship between size and stage of development for cysts of this type is shown in Fig. 1.

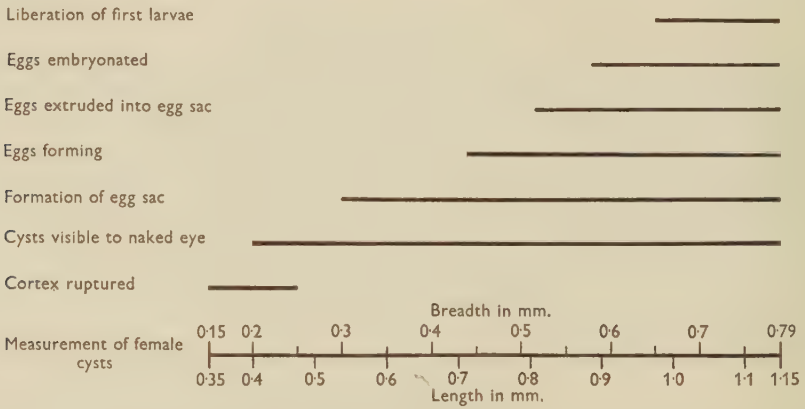

Liberation of first larvae
Eggs embryonated
Eggs extruded into egg sac
Eggs forming
Formation of egg sac
Cysts visible to naked eye
Cortex ruptured
Breadth in mm.
Measurement of female cysts
0·15 0·2 0·3 0·4 0·5 0·6 0·7 0·79
0·35 0·4 0·5 0·6 0·7 0·8 0·9 1·0 1·1 1·15
Length in mm.

Fig. 1. Relationship between size and stage of development in beet-eelworm cysts which suffer no check to growth.

The size range observed agrees closely with that obtained for brown cysts during previous soil-population studies (Jones, 1945). Cysts arrested in growth continue to pass through the successive developmental stages internally, except that the very smallest cysts, which have just ruptured the root cortex, may fail to produce eggs. Only the largest cysts succeed in extruding eggs into the gelatinous egg sac attached to the vulva. As a result of these preliminary studies measurement was discarded as a basis for the assessment of the rate of development and reliance was placed upon the easily determined developmental stages listed below:

(1) (Seed sown)	—
(2) Germinated	The first appearance of the cotyledons: corresponds to invasion by the first larvae
(3) Cystic forms	Swollen third stage larvae present in the roots
(4) Cortex ruptured	Cysts become visible to the eye with the aid of a × 6 hand lens.
(5) Eggs forming	The first appearance of eggs in the oviducts
(6) Eggs embryonated	The appearance of coiled embryos in the oldest eggs.

An additional seventh stage could be recognized, that of the emission of larvae from the cysts containing fully embryonated eggs. In practice, however, this stage was difficult to observe. Active larvae were sometimes found within the cysts or escaping from the gelatinous egg sac during late June, July and August. In some cysts, the presence of empty egg cases suggested that larvae had escaped. Mature white cysts placed in tap water at room temperature liberated larvae within a week. Although

there was no doubt that larvae were being liberated in the field within about 14 days of the embryonation of the eggs during July and August, the difficulty of observing the act made it impossible to use this stage. Confusion was also likely to arise from the ease with which ripe embryos escaped from embryonated eggs upon application of pressure during manipulation of cysts. Browning of the cysts appeared to bear no special relationship to the stage of development. It occurred at any stage upon the death of the female eelworm.

Observations

Using the six stages listed above, and confining observations to the largest cysts produced by the first larval invasions of each sowing, it was possible to draw curves showing the rate of development of cysts throughout the growing season (Fig. 2). The curves obtained from sugar beet, coleseed and mustard were coincident except for sowings made in late August. In these, the sugar-beet seedlings were adversely affected by early autumn ground frost. The coleseed and mustard (Fig. 2, curve *a*) were less affected and upon them cyst growth proceeded further than on sugar beet until falling soil temperatures brought cyst development to a standstill on all sowings.

In 1945, the field observations on beet eelworm were repeated using the same site. Fortnightly sowings of sugar beet were made from February to October (Fig. 3). Similar observations were also made on potato-, pea- and carrot-root eelworms using the same sites as in the host-range trials (Figs. 4–6). Except in the case of pea eelworm, the same developmental stages were utilized as for beet eelworm. With pea eelworm, slight modification was necessary because of the greater thickness of the pea tap root which results in pea eelworm cysts rupturing the cortex in a more advanced stage. For potato eelworm, time of germination was replaced by the time at which rootlets were put out by the potato sets. The sites for the beet-, carrot- and potato-eelworm observations were all on fen soils and situated within $1\frac{1}{2}$ miles of each other. Recording thermometers were not available to follow temperatures in the surface layers of the soil. Instead, on the site of the beet-eelworm trial, a soil thermometer was inserted to a depth of 2 ft. Conway (1936) has shown that for moist fen soil covered by fen litter, the large diurnal fluctuations in soil temperature scarcely penetrate below 1 ft. At 2 ft., therefore, in well-drained fen soil without a cover of vegetation, the soil temperature read at weekly intervals could be expected to give some indication of the general drift of soil temperatures throughout the season (Fig. 3). Throughout the rising phases of the curve, the mean temperatures at a depth of 3–6 in. are some degrees above that of the subsoil. The reverse holds for the falling phases of the curve. In addition to the temperature record at the beet-eelworm site, soil samples were taken weekly and dried at 100° C. to obtain a measure of the soil moisture content (Fig. 3). The pea-eelworm observations were made on peas sown on heavy land. Information about soil temperatures at this site were obtained from a weather station $\frac{1}{2}$ mile away. The drift of soil temperature at 2 ft. was almost identical with that at the Fenland site. On the fen soils, lack of moisture

was never a limiting factor for host plants or nematodes but, on the heavy land, the soil was hard and cracked in April. Half the first sowing of peas was watered and half left unwatered (Fig. 5, curves *a* and *b* respectively). In the watered half, invasion

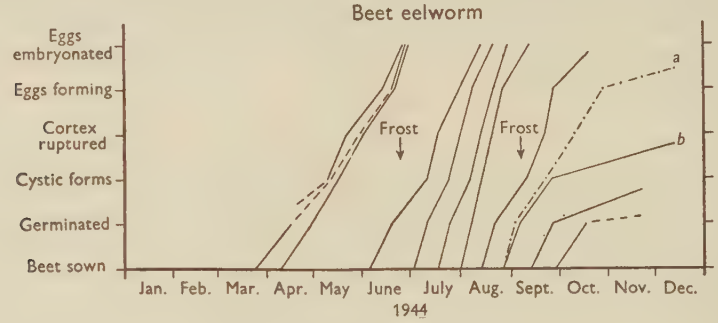


Fig. 2.

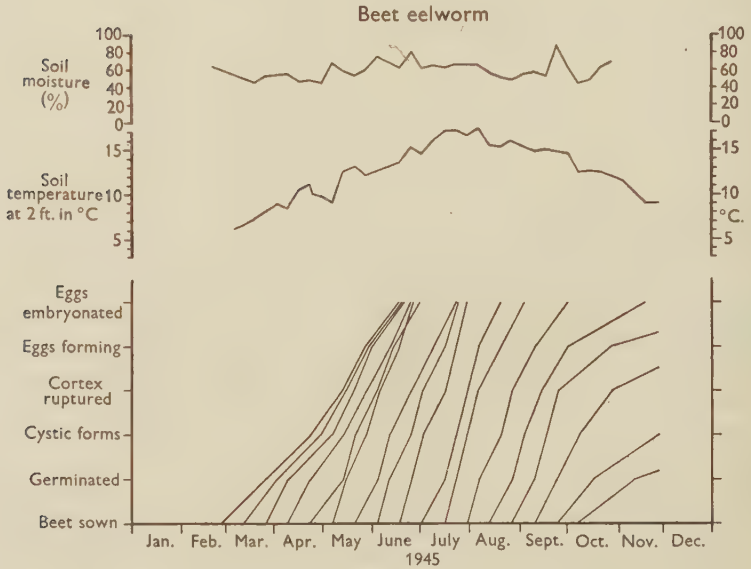


Fig. 3.

Fig. 2. Rate of development of beet-eelworm cysts in the field at a depth of 3–6 in.; year 1944. Host plants sugar beet, coleseed and white mustard. Curve *a*, coleseed and mustard only; curve *b*, sugar beet only.

Fig. 3. Rate of development of beet eelworm cysts in the field at a depth of 3–6 in.; year 1945. Host plant sugar beet. The upper curve indicates changes in soil moisture content (samples taken at weekly intervals). The middle curve shows the drift of soil temperature (weekly readings at a depth of 2 ft.). Mean soil surface temperatures are higher in the rising phases of the curve and lower in the falling phases.

of the rootlets and cyst development began at once, whereas in the unwatered half, invasion and development was delayed until rain occurred in May. Soil moisture again became limiting in July and August (Fig. 5, curves *c* and *d*). Light showers were sufficient to induce germination of the peas, but invasion of rootlets was delayed

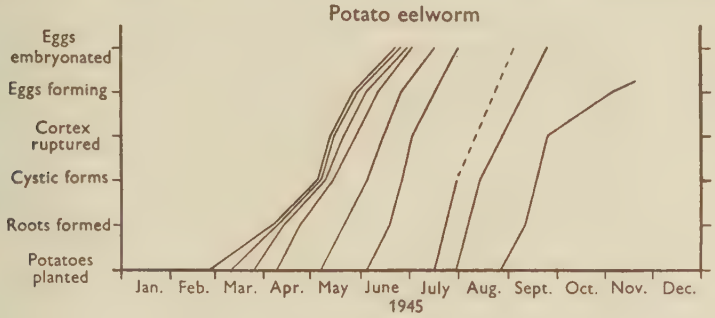


Fig. 4.

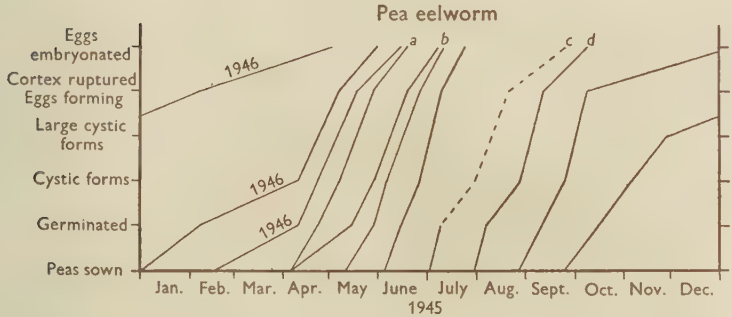


Fig. 5.

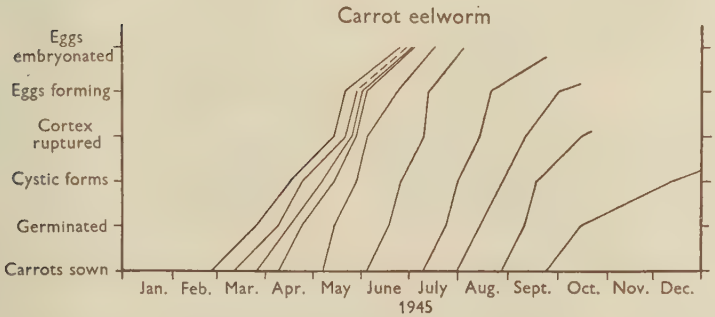


Fig. 6.

Figs. 4–6. Rates of development of potato-, pea- and carrot-eelworm cysts in the field at a depth of 3–6 in. Fig. 5, curve *a*, peas watered; curve *b*, peas unwatered; *c* and *d*, invasion of rootlets delayed by drought and, in *c*, the weight of the attack reduced.

and infestations were lighter than in the earlier sowings. At this site, the land was left unploughed and it was possible to continue observations into the following year (1946).

Interpretation of observations

The results obtained from the observations upon beet, potato and carrot eelworms on fen soils, where moisture was never limiting, and with pea eelworm, allowing for the effects of drought, show that the rate of development of cysts is closely related to soil temperatures. The rate is slow in early spring but increases as soil temperatures rise, reaching a maximum in July coincident with the highest yearly soil temperatures. In the autumn, as soil temperatures fall, so the rate of development slows down, becoming negligible in the winter months from November to March.

The curves also illustrate what happens to successive larval invasions of the roots of a host crop grown under normal farming conditions and indicates the possible number of generations of eelworms per year. This is determined by the length of vegetative period of the host crop and by climatic conditions through their effect upon soil moisture and temperature. The average crop of sugar beet occupies the land from April to November. Referring to Figs. 2 and 3, and allowing 14 days for the liberation of the first larvae from embryonated eggs in July and August, it appears that two and a half generations of beet eelworm were possible in 1944 and almost three in 1945. For a sugar-beet seed crop for which mother seed is normally sown in July, one and a half to two generations would be completed in the steckling bed before planting out in March of the following year, while a further two genera-

TABLE 4. *The possible number of generations of root eelworms on some common host crops*

Species of eelworm	Host crop	Vegetative period	Possible number of generations
Beet	Sugar beet	April–November	2½–3
	Sugar-beet seed:		
	Steckling stage	July–March	1½–2
	Seeding stage	March–August	2
	Black mustard	March–August	2
	Brussel's sprout:		
	Before transplanting	March–June	1
	After transplanting	June-March	2
	Winter rape for seed	July–July	3–4
	Catch crop, mustard or coleseed	July–October	1–1½
Potato	Potato:		
	Earlies	March–June	1
	Main crop	April–August	2
Carrot	Carrot:		
	Earlies	March–July	1
	Maincrop	May–November	2
Pea	Peas	March–July	1
	Spring beans	March–July	1
	Winter beans	October–July	2

tions would be possible in the seeding stage between March and August. Similarly, the number of generations possible can be determined for the other species of eelworm on various host crops (Table 4).

The number of generations deduced in this way is a maximum. In the field, although the first invasions by larvae begin with germination, the main invasions occur somewhat later when the root system has had time for further growth. Drought also would tend to limit eelworm activity and reduce the number of generations. The effects of drought would normally be most pronounced in July and August, the period of most rapid cyst development. Hellinga (1941) refers to observations by Backelandt and himself, in which beet plants were lifted at weekly intervals and the percentage of roots bearing white cysts was recorded. The percentage of such roots rose to a maximum in the third week in June with a suggestion of a second peak in August, although the later observations were not so thorough as the earlier ones. In July there was a period when the percentage of roots bearing white cysts fell to zero. Further observations in other fields, however, gave no evidence of periodicity of beet-eelworm attack and root examination indicated that invasion was more or less continuous. During eelworm surveys in East Anglia (Petherbridge & Jones, 1944), the author found little evidence of periodicity in most beet fields in the fens and it was possible to continue survey work from June to October. There were, nevertheless, a few fields in which there was difficulty in finding cysts in July and these appeared to be suffering from drought.

Abundant proof that two and a half generations of beet eelworm may occur between April and November was obtained during the beet-eelworm host-range trial in 1945. In July, mature white cysts were collected from the roots of twenty-nine different host plants (Table 3). These were transferred to pots of sterilized soil and planted with sugar beet. The pots were kept out of doors sunk in horticultural peat and watered liberally. On being turned out in late August, second generation cysts were found on the plants in all but one pot. Mature white cysts were collected from the sugar-beet plants and placed in pots of sterilized soil. Beet sown in these pots bore immature white cysts of the third generation in November. Sugar beet sown in sterilized soil alone remained free from cysts.

Similarly, pot tests were carried out with potato, carrot and pea eelworms. Second generations were obtained on potatoes and carrots but not on peas. With potato eelworm, although there is time for two generations on the main crop, it is not certain that a full second generation occurs in the field. By July, main-crop potatoes have usually passed the peak of vegetative growth, and after this the root secretions may possibly become less active with a corresponding reduction in the hatch from cysts. Observations on the *Galeopsis* eelworm suggest that it behaves in much the same way as beet eelworm. Larvae hatch freely in tap water and can sometimes be observed escaping from the gelatinous egg sac. On suitable host plants it would probably give rise to between two and three generations per year.

The above observations have a bearing upon trap cropping as a possible control measure of root eelworms and upon the status of catch or cover crops in areas affected by beet eelworm. Setting aside the technical difficulties which make trap cropping almost impossible to carry out in practice, it can be seen that for beet, potato, carrot and pea eelworms, a trap crop sown in early April would have to be destroyed within 8–9 weeks, while trap crops sown in June or July would have to be destroyed within 5–6 weeks of sowing. The common cover crops utilized after early potatoes or early carrots are mustard and coleseed. In July and August, beet eelworm cysts forming on the rootlets of such crops would begin egg production in about 5 weeks from germination. Generally, cover crops are left in for a much longer period and, in areas infected with beet eelworm, they must be regarded as playing a part in the general increase of populations. An immune cover crop to replace coleseed and mustard is highly desirable.

Cyst morphology

During the host-range trials and the observations on the rate of development of cysts, fresh cysts of the various species of *Heterodera* were examined in large numbers, and outline drawings were made with a camera lucida at all stages in development. A representative series of outline drawings is shown in Fig. 7. In these diagrams, differences in the shape and in the size ranges of the cysts of the various species are apparent, and these and other characters are considered briefly in turn.

Shape

Differences in cyst shape are more clearly visible in large cysts than in the smaller immature stages. The cysts of the potato eelworm, with their characteristic subspherical outline, are readily separated from the other species all of which have lemon-shaped cysts. Amongst these, the cysts of the *Galeopsis*-root eelworm most nearly approach the potato eelworm in shape but differ in the possession of a small vulva. The vulva is very prominent in the beet eelworm and much less prominent in the other lemon-shaped species, especially cereal- and hop-root eelworms.

Egg sac

The presence of a gelatinous egg sac appears to be a constant feature of all the species of *Heterodera* under observation, except for potato-root eelworm in which it is never present. When roots bearing cysts are lifted the egg sac tends to dry out and is easily lost. Sometimes it adheres firmly to the host rootlet near the hind-end of the cyst and is left behind when the cyst is removed. Very often it is entirely obscured by grains of soil which stick to it.

The egg sac begins to form shortly after the cyst has ruptured the root cortex. In the pea eelworm, owing to the thickness of the pea and bean tap-roots, formation of the egg sac sometimes begins while the cyst is wholly within the cortex. As cysts grow, eggs may be extruded into the egg sac and, on this basis, a series may be drawn

up beginning with *H. marioni* where eggs are usually extruded immediately after they are formed and ending with *H. rostochiensis* in which there is no egg sac and all the eggs are retained within the body of the female:

H. marioni	All eggs extruded into egg sac
H. carotae, *H. cruciferae*	Egg sac almost as large as the cyst, half the eggs extruded
H. göttingiana	Variable, moderate number of eggs sometimes present in the egg sac (possibly affected by drought)
H. schachtii, *H. galeopsidis*	Extrusion of eggs occurs only in the largest cysts, few eggs extruded
H. major, *H. humuli*	Egg sac always present, no eggs extruded
H. rostochiensis	No extruded eggs and no egg sac

In all species except the potato eelworm, males are found in a small percentage of the sacs throughout all stages of growth. Sometimes more than one male is present. Most commonly the male lies curved within the egg sac from which it often emerges when the cyst is placed in tap water. Occasionally a male is found tightly coiled about the vulva of the female.

Coloration

The cysts are ivory white when they first rupture the cortex of the root which they have invaded. In all but the *Galeopsis* and potato eelworms, this colour is retained until the cyst wall turns brown. In the *Galeopsis* and potato eelworms, the cysts change colour to yellow before maturity is reached, and it is in this state that they are commonly observed when infected plants are lifted in the field. The yellow colour does not reside in the cyst wall. If a cyst of either species is punctured the colour flows out with the body contents leaving behind the almost transparent cuticle. The similarity in colour between *Galeopsis* and potato-eelworm cysts, together with the general similarity in shape, might lead to confusion of the species and may be responsible for some of the records of potato eelworm occurring upon sugar beet and upon other weeds.

Size ranges and length-breadth relationships

Cysts of the eight species of eelworm in Fig. 7 were collected from the appropriate host plants and measured under a low-power monocular microscope with the aid of a calibrated eyepiece. The measurements made were length, including the neck, and breadth. Accurate measurement is not possible because most cysts are asymetrical in shape and because, under the microscope, they rarely lie with their axes at right angles to the optical axis. Measurements were made in all stages of cyst growth so as to cover the length-breadth relationships from the rupture of the root cortex up to the maximum size attained.

The average size of a cyst population is not of great diagnostic value, since the average varies considerably with the conditions under which the population was

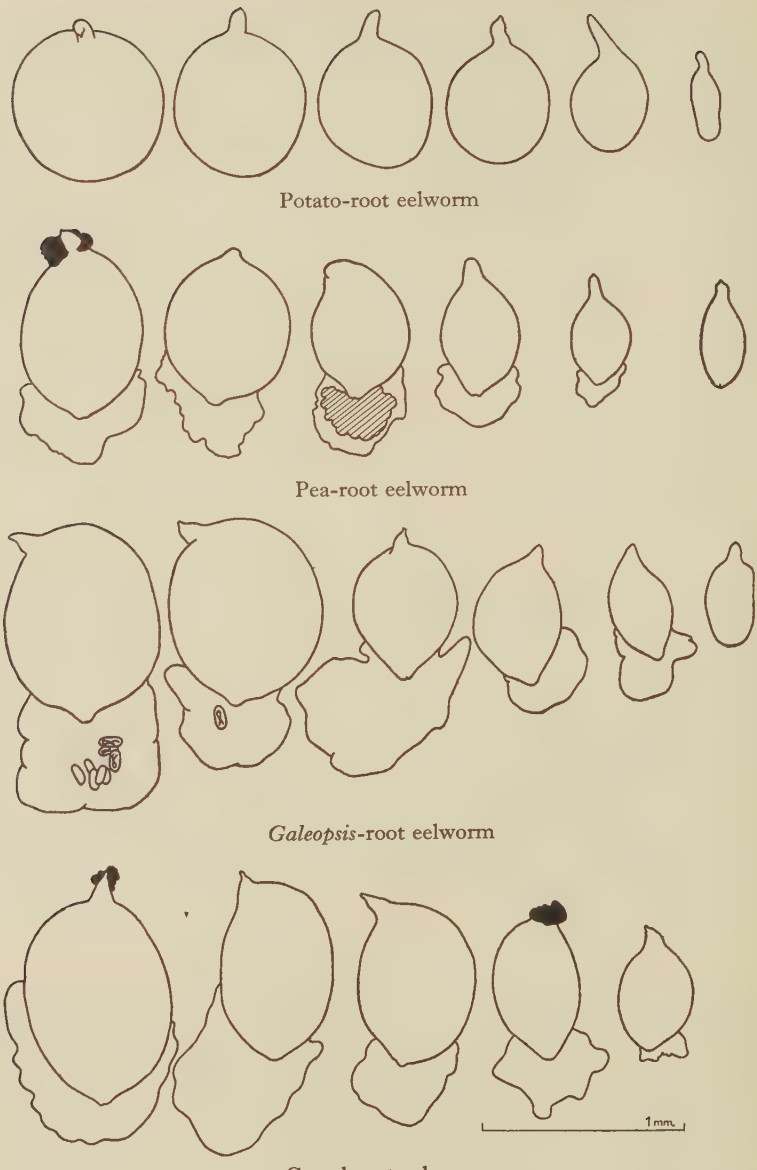
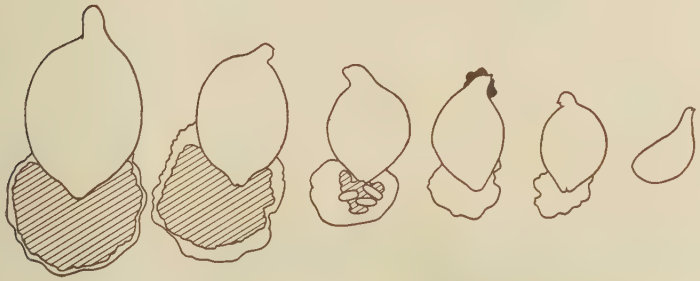


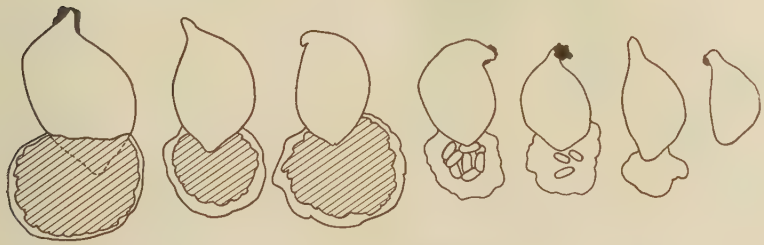
Fig. 7. Outline diagrams to illustrate the shape and size range of various root eelworm cysts, the form of the egg sac and the extrusion of eggs. The dark substance adhering to the cysts is a viscid secretion produced especially near the head. Where the egg mass in the gelatinous sac is opaque, the space occupied by eggs is shaded.

Fig. 7 (*continued*)

Beet eelworm



Cabbage-root eelworm



Carrot-root eelworm

Hop-root eelworm

produced. Thus a beet crop lifted in December would probably produce many more small third generation beet-eelworm cysts than a crop lifted in September. Of somewhat greater value than average size is the size range for any species. The size ranges observed are set out in Table 5. The *Galeopsis*, beet, oat and potato eelworms reach approximately the same maximum size, although the beet eelworm seems relatively longer and thinner than the other species. Carrot eelworm has the smallest maximum size. Minimum sizes are to some extent determined by the root thickness of the host plant. Pea eelworm, growing in the rather thick roots of peas and beans (*Vicia*), has the largest minimum size, while hop eelworm has the smallest. Potato eelworm cysts are also rather small when they first rupture the cortex of potato rootlets.

TABLE 5. *Size ranges of eelworm cysts in* 100*th of a mm.*

Species of eelworm	Maxima		Minima		Range	
	Length	Breadth	Length	Breadth	Length	Breadth
Beet	115	79	35	15	80	64
Galeopsis	100	85	45	22	55	63
Potato	100	86	34	10	66	76
Cereal	100	80	—	—	—	—
Pea	94	78	(40)	(25)	(54)	(53)
Cabbage	83	57	35	15	48	42
Hop	76	60	28	10	48	50
Carrot	68	53	21	15	47	38

TABLE 6. *Regression coefficients calculated from length and breadth measurements of eelworm cysts collected from the roots of host plants*

Species of eelworm	Number measured	Regression coefficient, length/ breadth	Mean coefficient (%)	Regression coefficient, breadth/ length	Mean coefficient (%)
Beet	452	0·9484	111·1	0·7222	89·8
Carrot	254	0·6584	77·1	0·7062	87·9
Galeopsis	51	0·8503	99·6	1·0035	124·9
Cabbage	88	0·8736	102·3	0·8624	107·3
Potato	104	0·7198	84·3	1·1577	144·0
Pea	77	1·1401	133·6	0·6635	82·5
Oat	54	0·8159	95·6	0·9315	115·9
Hop	235	0·8943	104·8	0·8621	107·3
	Total 1315	Mean 0·8536	100·0	0·8038	100·0
		S.E. ±0·059	± 6·9	±0·056	± 7·0
	Sign. diff.		± 13·8		± 14·0

The relationships of the length and breadth measurements of the various species are shown in Fig. 8. Length/breadth ratios of cysts are sometimes quoted in the literature, often based on relatively few measurements. The validity of such a measure is open to question since the ratio obtained varies with the stage of growth of the cysts. Thus, for beet eelworm the length/breadth ratio of the smallest cysts is approximately 2·0, while for the largest cysts, it is of the order of 1·4. The length and breadth relationships are expressed more accurately by the regression coefficients, and these are tabulated in Table 6. In calculating the coefficients, it was first

ascertained that for beet-eelworm cysts, there was no significant difference in the coefficients of measurements made in 1944 compared with those of 1945, nor was there any significant difference between the coefficients of cysts taken from sugar beet, coleseed or mustard.

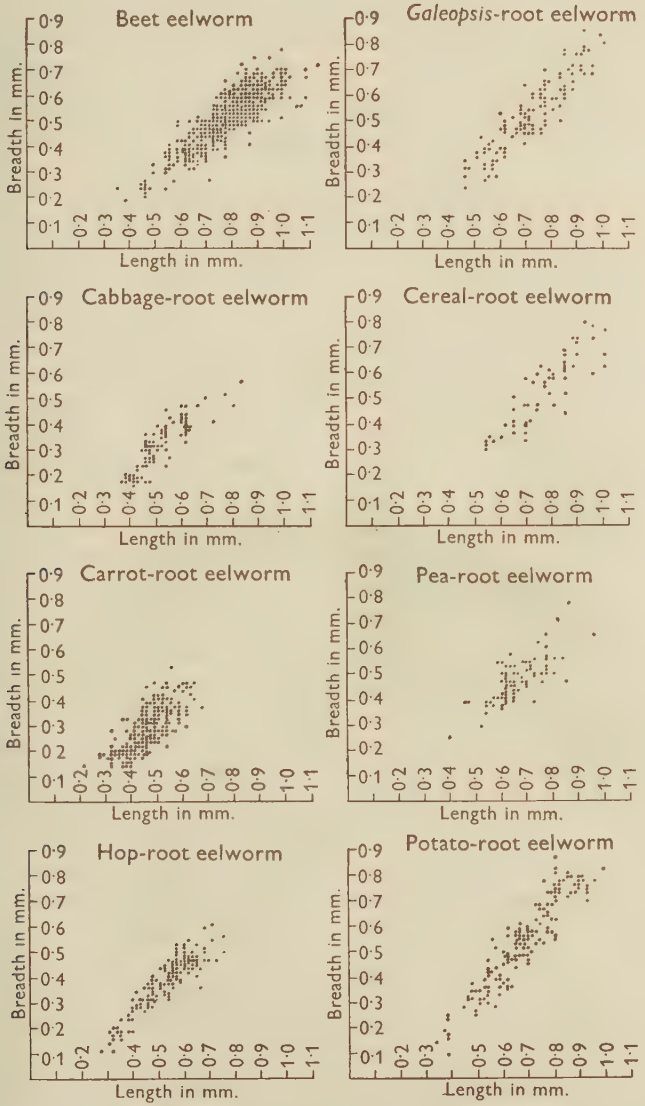


Fig. 8. The length-breadth relationships of eight species of root eelworm, compiled from measurements of cysts taken from the roots of their respective host plants.

The differences in the length-breadth relationships between the cysts of the various species of eelworm are not sufficient to warrant the calculation of a discriminative function. Nor are they great enough to be of much assistance in separating mixed populations of lemon-shaped cysts from soil samples, although they are of some value in characterizing cysts collected directly from host plants. It remains to be seen whether similar values for the regression coefficients would be obtainable from cysts derived from other localities. It is conceivable that certain types of soil, especially the heavier types, might impose a restraint on the free growth of cysts and thus cause variation in shape and size.

Two little-known species of Heterodera

The carrot-root eelworm (Heterodera carotae *Jones*)

The earliest record of a root eelworm producing cysts on carrots appears to be that of Triffit (1935). The species has been partly described (Jones, 1950) and a more complete description is given below in Table 7. *H. carotae* falls into the group of *Heterodera* species in which the male has copulatory spicules with bi-dentate tips, as in *H. schachtii* and *H. cruciferae*. On this character alone it can be distinguished from the remaining species. From *H. schachtii*, it differs considerably in size and shape, and in the possession of a large egg sac into which many eggs are extruded, and also in host range. From *H. cruciferae*, it may be separated by its smaller size (Figs. 7, 8), by significant differences in its length-breadth relationships (Table 6), and by its host range. In addition, the larval length of *H. carotae* is significantly greater than that of *H. cruciferae* when measured by the standard technique of Fenwick & Franklin (1942) (see Table 8). These differences taken together are sufficient to merit specific status for the carrot-root eelworm.

In the summer of 1944, cysts of this root eelworm were found on carrots growing on a small-holding in the Chatteris area of the Isle of Ely. Subsequently, infestations were noted on several other holdings in the same area, some of which were reputed to have grown carrots for as many as 20 years in succession. Where the infestation was heavy, the crops were poor and patchy. Plants lifted from the worst patches were stunted and carried an abundance of lateral roots but this development of lateral roots was less marked than with sugar beet heavily attacked by the beet eelworm. The foliage in the poor patches had a reddish tinge, a symptom commonly occurring in carrots whenever the root system is impaired.

The Galeopsis-*root eelworm* (Heterodera galeopsidis = H. schachtii *var.* galeopsidis *Goffart*)

The *Galeopsis*-root eelworm was found in 1939 when examining weeds for beet eelworm in the Sutton area of the Isle of Ely. In this locality, the crop rotation is based chiefly on cereals, potatoes, sugar beet and carrots. Clovers and other leguminous crops are rarely grown. The eelworm appears to persist chiefly on four

TABLE 7. *Description of the carrot-root eelworm,* Heterodera carotae *Jones*

Cysts:		
Shape	Lemon (Fig. 7)	Based on examination and measurement of 254 cysts from the roots of carrots
Size:		
Length	Max. 0·68 mm., min. 0·53 mm. (Table 5)	
Breadth	Max. 0·21 mm., min. 0·15 mm. (Table 5)	
Length/breadth	2·3 to 1·35	
Regression coeffs.:		
Length/breadth	0·6584 ± 0·059 (Table 6)	
Breadth/length	0·7062 ± 0·056 (Table 6)	
Colour	White, changing to brown without intermediate stage	
Gelatinous egg sac	Large. Begins forming when the cysts are approx. 0·40 mm. × 0·2 mm.; in fully grown cysts, equal in size to the cyst itself (Fig. 7).	
Extruded eggs	Many (Fig. 7)	
Punctations	Irregular	
Subcrystalline layer	Present	
Eggs:		
Size:		100 eggs measured, 10 from each of 10 cysts
Length	103·4 ± 0·44 μ	
Breadth	46·7 ± 0·31 μ	
Length/breadth	2·21	
Larvae:		
Length	453·8 ± 1·38 μ	100 larvae measured, 10 from each of 10 cysts, using technique of Fenwick & Franklin, 1942
Tail	Pointed	
Male cysts:		
Size on rupturing cortex	0·33 × 0·09 mm. approx.	13 specimens
Males:		
Size:		
Length	1·19 ± 0·014 mm.	25 specimens
Breadth	31·4 ± 0·6 μ	25 specimens
Stylet	28·8 ± 0·36 μ	30 specimens
Copulatory spicules	Bi-dentate as in *H. schachtii*	—
Host range	Only carrot and wild carrot attacked out of 105 plants tested (Table 1)	—

common weeds: chickweed (*Stellaria media* (L.) Vill.), the hemp nettles (*Galeopsis tetrahit* L. and *speciosa* Mill.), and procumbent speedwell (*Veronica agrestis* L.). These weeds are most abundant in cereal crops, but they also grow to some extent in the root crops. It is possible that the eelworm also persists on sugar beet, although this plant and its allies do not seem to be efficient hosts. In eelworm surveys in the Sutton area (Petherbridge & Jones, 1944), many fields were examined for the presence of eelworm and, although a considerable number of fields were found infected with beet eelworm, *Galeopsis* eelworm was not observed on sugar beet or mangolds. Only in the host-range trial of 1945 was there any indication that sugar beet and its allies might serve as hosts. Both beet and *Galeopsis* eelworms have been

afforded opportunities for increase in the area by frequent cropping with sugar beet, mangolds and red beet, but only beet eelworm appears to have spread appreciably in the last decade. The economic importance of the *Galeopsis* eelworm, therefore, lies chiefly in its possible confusion with beet eelworm, because of the overlap in host range, and with the potato-root eelworm because of the colour and rotundity of its cysts.

TABLE 8. *Comparison of the larval lengths of* Heterodera carotae *and* Heterodera cruciferae

Species	Larval length (μ)	Standard error (μ)	Number measured
H. carotae	453·8	±1·38	100
H. cruciferae	417·9	±0·519	1850 (Franklin, 1945)
Difference	35·9	±1·2	1950

$t=30$, difference significant beyond $P=0{\cdot}001$.

TABLE 9. *A comparison of beet eelworm with* Galeopsis*-root eelworm*

	Beet eelworm (*H. schachtii* Schm.)	*Galeopsis*-root eelworm	Remarks
Cysts:			
Shape	Lemon, long	Lemon, round	Fig. 7
Size: Length	Max. 1·15 mm.	Max. 1·00 mm.	Table 5
	Min. 0·35 mm.	Min. 0·45 mm.	
Breadth	Max. 0·79 mm.	Max. 0·85 mm.	
	Min. 0·15 mm.	Min. 0·22 mm.	
Length/breadth	2·0 to 1·4	2·0 to 1·2	
Regression coeffs.:			Table 6
Length/breadth	0·9489	0·8503	Difference insignificant
Breadth/length	0·7222	1·0035	Difference significant at $P=0{\cdot}001$, $t=5$
Number measured	452	51	
Colour while living	White throughout life	White at first, then pale yellow	
Egg sac	Present	Present	
Extruded eggs	A few eggs extruded by the largest cysts only	A few eggs extruded by the largest cysts only	
Punctations	Irregular	Irregular	
Subcrystalline layer	Present	Present	
Eggs:			
Size:			
Length	$111{\cdot}4\,\mu\pm0{\cdot}6$	$108{\cdot}1\,\mu\pm1{\cdot}0$	Difference significant, $t=3$, $P=0{\cdot}01$
Breadth	$42{\cdot}0\,\mu\pm0{\cdot}2$	$47{\cdot}3\,\mu\pm0{\cdot}44$	Difference significant, $t=5$, $P=0{\cdot}001$
Length/breadth	2·65	2·29	
Number measured	100	50	
Larvae	(Franklin, 1945)		
Length (standardized technique)	$470{\cdot}6\,\mu\pm0{\cdot}602$	$521{\cdot}2\,\mu\pm13{\cdot}2$	Difference significant beyond $P=0{\cdot}001$, $t=12$
Number measured	1360	40	
Tail	Pointed	Pointed	
Males:			
Spicules	Bi-dentate		

The relationship of the *Galeopsis* eelworm to other 'races' and species of *Heterodera* attacking sugar beet, hemp nettle and clovers must now be considered. In Table 9, the beet and *Galeopsis* eelworms are compared. They are similar in the irregular punctation of the cyst wall, in the possession of a subcrystalline layer and in the form of the egg sac and the number of extruded eggs. They differ in the colour and shape of the cysts, in the regression coefficients of breadth on length, in larval length and in the dimensions of the eggs. The two eelworms are very similar in biology. The larvae hatch readily from cysts placed in tap water and their host ranges overlap but, despite this overlap, as already pointed out, they behave very differently towards sugar beet. The differences between these two forms appear to warrant the placing of the *Galeopsis* eelworm outside the species *Heterodera schachtii*.

The only previous record of a root eelworm attacking hemp nettle (*Galeopsis tetrahit* L.) appears to be that of Goffart (1936), although Hollrung (1890) noted cysts on *Lamium amplexicaule* L., and Triffit (1929) recorded cysts on *L. purpureum* L. which are also members of the family Labiatae. In the host range trial in 1945 (Table 1), *L. purpureum* L. was not attacked, a result in agreement with the original observations of Goffart. There are several previous observations of root eelworms attacking clovers, the most important being those of Goffart (1932) and Franklin (1939, 1945). The question arises whether the present eelworm on *Galeopsis* spp. is the same as the one observed by Goffart (1936) and also, what is its relationship to that attacking clovers? The available information is set out in Table 10. The information given by Goffart on the *Galeopsis* eelworm is rather meagre. It offers no comment on the colour of the cysts except that they are brown in October. No details are given of the egg sac or the subcrystalline layer. The measurements of cysts, eggs and larvae are not inconsistent with those given for *Galeopsis*-root eelworm in Table 9. There are, however, two positive characters which suggest that the two forms are the same. The first is the rotund shape of the cysts, of which outline diagrams are given in Goffart's text, and the second is the fact that both forms are strongly parasitic on hemp nettle, weakly or non-parasitic on sugar beet, and non-parasitic on red dead nettle, oats and potatoes. Much the same remarks apply in comparing the *Galeopsis* and clover eelworms, although here more information is available. The measurements of the cysts, eggs and larvae are not inconsistent with those given for *Galeopsis* eelworm in Table 9. The coloration of the cysts is the same, but cyst shape appears to be different and, moreover, the clover eelworm is strongly parasitic on clovers, whereas the *Galeopsis* eelworm is apparently not. Apart from cyst shape and host range and the fact that Goffart considered the hemp nettle and clover eelworms to be distinct varieties, there are no other characters available on which to decide the status of the eelworm attacking *Galeopsis* spp. in the Sutton area. It therefore seems best, for the present, to identify this form with the var. *galeopsidis* of Goffart (1936), and in view of the differences between it and the beet eelworm, to accord it specific status under the name *Heterodera galeopsidis*, the *Galeopsis*-root eelworm. Further work may prove or disprove the validity of this step.

Table 10. *Details of* Heterodera schachtii *var.* galeopsidis *Goff. & var.* trifolii *Goff.*

	H. schachtii var. *galeopsidis* Goffart, 1936	*H. schachtii* var. *trifolii* Goffart, 1932
Cysts:		
Shape	Lemon, round	Lemon, medium (Franklin, 1939)
Size:		
Length	Max. 0·905 mm. Min. 0·453 mm.	— —
Breadth	Max. 0·662 mm. Min. 0·313 mm.	Max. 0·525, 0·550 mm. Min. 0·250, 0·225 mm. (Franklin, 1939)
Number measured	(78)	(100) (100)
Length/breadth	1·45 to 1·37	2·4 to 1·3 (Goffart, 1936)
Colour (while living)	? (brown in October)	White at first, then pale yellow
Egg sac	?	Present (Goffart, 1936; Franklin, 1939)
Extruded eggs	?	A few eggs extruded by the largest cysts only (Franklin, 1939)
Punctations	?	Irregular
Subcrystalline layer	?	Present (Franklin, 1939)
Eggs:		
Length	108 μ (100)	118·8 μ ± 0·55 (50) (Goffart, 1936) 114 μ (10) (McBeth, 1938) 98 μ (100) (Franklin, 1939)
Breadth	44·5 μ (100)	49 μ (10) (McBeth, 1938) 40 μ (100) (Franklin, 1939)
Length/breadth	2·42 (100)	2·33 (10) (McBeth, 1938) 2·45 (100) (Franklin, 1939)
Larvae:		
Length (measured by unstandardized technique)	485 μ (100)	401 μ (120) (Goffart, 1936) 480 μ (10) (McBeth, 1938) 491 μ (100) (Franklin, 1939) 529 μ (300) (Franklin, 1940)
Males:		
Spicules	?	Bidentate (Franklin, 1945)
Hosts:	*Galeopsis tetrahit* L. Not sugar beet, oats, potato or *Lamium purpureum* L.	Red Clover (Goffart, 1936) White Clover (McBeth, 1938) Clovers and docks attacked heavily Mangold, sugar beet and garden pea attacked only with difficulty (Franklin, 1945).

Conclusion

In the literature upon root eelworms, the theory of the adaptability of the so-called strains or biological races of *Heterodera schachtii* sensu lat. has been widely held until the last decade. The present observations tend to support the opposing theory, that of the fixity of the strains and their recognition as distinct species. It seems unlikely that forms with limited host ranges such as the potato, cereal, pea, carrot and cabbage-root eelworms would be able to adapt themselves readily to new hosts.

Moreover, there are valid reasons in each case for regarding them as distinct species. The position is not so clear with forms such as the beet-, clover- and *Galeopsis*-root eelworms which possess wide host ranges overlapping to some extent. In the present host range experiments and in field observations, there has been no evidence that the beet eelworm is variable. It appears to have the same host range amongst common weeds and crop plants throughout the infected area in the Fens and also in other isolated localities where it occurs. Further, the host range appears to be the same whether the effective population is built up on sugar beet, mangolds or Cruciferous crops. It is not possible to speak with the same force regarding the clover and *Galeopsis* eelworms, but these do appear to be distinct from the beet eelworm, if only in the yellow intermediate colour of the cysts.

Assuming the theory of fixity of species to be true, the sudden and unexplained appearance of devastating populations of root eelworms in new localities would be attributed to a definite species already in existence and not to the relatively rapid adaptation of *Heterodera schachtii* sensu lat. In nature, cyst-forming species of *Heterodera* are inconspicuous, their high reproductive capacity being largely off-set by the difficulties of finding hosts. Farming and horticulture provide opportunities for multiplication, especially where susceptible crops are grown frequently without regard to crop rotation, while commerce has broken down geographical barriers, thus ensuring the dissemination of cysts over the cultivated areas of the earth.

Although the above view of the origin of root eelworms in crops seems the most likely, it would be unwise to deny altogether the possibility of adaptation to new hosts. Adaptation must have occurred during the course of evolution and may be continuing, though probably at a much slower rate than envisaged by the exponents of the theory of adaptability.

I am indebted to Mr F. R. Petherbridge under whose direction the work was begun, to Dr Wishart for advice on statistics and to my wife for reading through the script.

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INFESTATION OF A BEAN FIELD BY *APHIS FABAE* SCOP. IN RELATION TO WIND DIRECTION

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(With Plate 13 and 3 Text-figures)

The wind direction during the primary migration of *Aphis fabae* Scop. gave a plausible explanation of the infestation pattern round the edge of a bean field; but it is emphasized that other factors are involved whose effects have not been adequately assessed in this preliminary survey.

Introduction

There is a common belief that the infestation of a bean field by *Aphis fabae* Scop. often begins at the edges and that, although it may eventually sweep across the field, the edges usually suffer most.

Two questions arise: are the locations of the first large colonies directly associated with wind direction during primary migration? Can the final pattern of infestation be related to the wind direction during the times of maximum migration from the winter hosts?

Observations were made in 1948 on the following:

(1) The rate and date of departure of alate *A. fabae* from selected bushes of *Euonymus* and *Viburnum*.

(2) The rate and date of arrival of migrant aphids of the '*fabae* complex' on traps around a crop on Great Field 1 at Rothamsted and on a small plot of beans in a neighbouring garden.

(3) The general wind direction during primary migration was determined by means of phosphorus smoke liberated at Great Field and by a Dines's anemobiograph at the main laboratories, Rothamsted.

(4) The location of any large and fairly constant eddies around the crop.

(5) The subsequent pattern of infestation at the edges of the crop.

Primary migration from winter hosts to beans

The primary migration was studied by watching marked twigs of *Euonymus europaeus* and *Viburnum opulus* for the departure of *Aphis fabae* and noting the arrival of black aphids of the *fabae* complex on a small bean plot in a nearby garden and on sticky traps round the main crop.

Migration from selected plants

Selected twigs heavily infected with apterae were labelled and watched until the alatae began to appear, early in May. Counts of apterae and alatae were then made at least once and sometimes several times a day during May. The very crowded nature of the colonies and the reluctance to disturb them has led to some inaccuracy with the counts of apterae (see Tables 1 and 2). In spite of close observation, however, no very large, sudden, massed departures were detected, and the impression was formed that the flighting of alatae kept pace with their production from the winged nymphs.

TABLE 1. *Numbers of alate and apterous* (*bracketed*) Aphis fabae *on twigs of* Viburnum opulus *in a St Albans garden*, 1948

*=apterae, approx. nos.

Date (May)	Numbers on separate twigs							Total alatae
4	3 (89)	0 (24)	4 (75)	—	—	—	—	7
5	8 (73)	0 (13)	5 (78)	1 (25)	0 (34)	2 (12)	1 (38)	17
6	10 (70)	0 (11)	8 (95)	3 (30)	0 (39)	0 (13)	2 (45)	23
7	13 (74)	0 (12)	9 (105)	2 (25)	1 (36)	0 (16)	1 (54)	26
9	18 (45)	2 (7)	25 (200*)	1 (35)	9 (100*)	3 (100*)	17 (77)	75
10	22	0 (0)	38	2	8	5	21	96
11	14 (18)	—	46	5 (9)	6	8 (67)	16 (72)	95
12	7 (5)	—	—	3 (4)	14 (60*)	9 (150*)	—	33
14	1 (0)	—	57 (200*)	1 (0)	10 (57)	30 (126)	—	99
15	0 (0)	—	59 (70)	0 (0)	9 (70)	48 (83)	28 (100*)	144
16	1 (0)	—	33 (40)	1 (1)	10 (75)	10 (42)	21 (80*)	76
17	0 (0)	—	20	0 (1)	15	15	22	72
18	—	—	14 (30)	0 (0)	8 (26)	12 (26)	16	50
19	—	—	8 (11)	—	0 (0)	9 (8)	11 (47)	28
20	—	—	4 (2)	—	—	2 (9)	7 (45)	13
21	—	—	0 (0)	—	—	0 (3)	4 (53)	4
22	—	—	—	—	—	0 (0)	3 (34)	3
23	—	—	—	—	—	—	0 (0)	0

TABLE 2. *Numbers of alate and apterous* (*bracketed*) Aphis fabae *on twigs of* Euonymus europaeus *at Rothamsted*, 1948

Date	Numbers on separate twigs			Total alatae
19 May	18	22	17	57
20	17	45	28	90
21	23	19	38	80
22	21 (25)	54 (190)	9 (47)	84
24	5 (8)	59 (140)	11 (50)	75
25	2 (13)	56 (134)	12 (46)	70
26	0 (0)	55 (114)	14 (37)	69
27	0 (1)	59 (76)	7 (18)	66
28	0 (12)	1 (10)	38 (51)	39
31	0 (11)	1 (7)	0 (0)	1
1 June	0 (4)	0 (0)	1 (0)	1
2	0 (5)	0 (0)	0 (0)	0
7	Whole plant nearly free			—

In Tables 1 and 2 numbers of alatae and apterae in all stages are given once for each day. The count was made between 9 and 9·30 a.m. for the *Viburnum opulus* in a garden at St Albans, 4 miles distant (Table 1), and at 1–1.30 p.m. for the *Euonymus europaeus* at Rothamsted, $\frac{1}{4}$ mile to the north-east of the bean field (Table 2). The *Euonymus* at Hammond's End, 2 miles away to the south was visited usually between 3 and 4 p.m., but in spite of a good initial infestation there, no large colonies developed.

It will be seen that the changes in numbers are gradual over successive days. The fairly rapid disappearance of apterae towards the end of the migratory period was almost entirely due to predators and was not due to a production and sudden departure of alatae. Departure of alatae from the labelled twig, moreover, need not imply departure from the bush.

TABLE 3. *Daily arrival of alate black aphids* (fabae *complex*) *on small bean plot at Rothamsted Lodge garden,* 1948

(Aphids counted and removed once each day.)

Date	No. of plants examined	Total alatae	Alatae per plant
7 May	118	0	0
8	118	1	0·008
10	118	14	0·12
11	118	59	0·50
12	118	45	0·38
13	118	48	0·41
14	118	85	0·72
15	118	87	0·74
16	118	44	0·37
17	118	36	0·31
19	117	205	1·75
20	63	55	0·87
21	117	21	0·0*
22	117	108	0·92
24	32	68	2·13
25	117	222	0·33*
26	32	10	0·31
27	117	66	0·33*
28	116	38	0·33
29	90	19	0·21
31	32	17	0·35*
1 June	83	41	0·13*
3	118	27	0·18*
8	118	31	0·26
15	109	32	0·29

* Calculated on basis of uniform redistribution of uncounted, residual aphids of the previous day.

Influx of migrants on the crop

The garden plot. Field beans had been transplanted, early in April, to a plot 15 yd. square in a sheltered garden at Rothamsted Lodge, about 300 yd. to the

south-south-east of the main crop. Early in May, when migration started, the plants were 9–15 in. high and 3 ft. apart.

Every day, the same alternate plants were carefully examined and every alate black aphid removed and killed. It would have been better for these observations had all the plants in the plot been so treated; but the plot and its infestation were being used for another experiment. Each daily batch, therefore, particularly towards the end of May was made up partly from the influx of new migrants and partly from those moving across from other plants in the plot. In Text-fig. 3 and in Table 3 a correction has been made on the assumption that during the 24 hr. after hand-picking half the population on the remaining untouched plants moved over to the clean plants. This gives a graph underneath the observed one but it follows the same trend. The actual state of affairs is probably somewhere in between the two. Data are given in Table 3.

Traps at the main crop. Four sticky traps similar to those described by Broadbent, Doncaster, Hull & Watson (1948) were erected, one at the middle of each side of the main crop, on 27 April. Each trap was at the very edge of the crop, with its centre $2\frac{1}{2}$ ft. above the ground; i.e. with the trap just above crop level. A 9 in. suction trap with the rim $2\frac{1}{2}$ ft. above the ground was placed in the open space in the middle of the crop (Text-fig. 2).

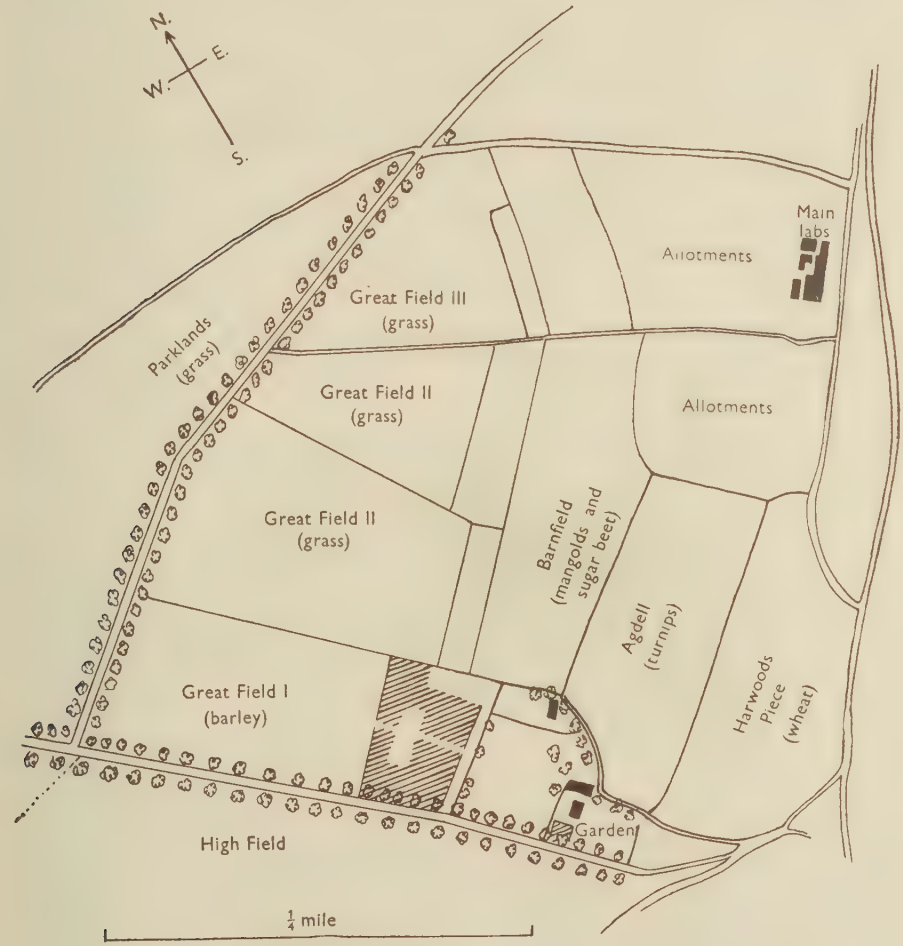
The results for all the traps are given in Text-fig. 3. The trap records, as a whole, help in estimating the start of the primary migration, but they do not elucidate the relative distribution of aphids on different sides of the field. The small catches may have been due to bad siting of the traps; and more traps should have been used.

The infestation of the bean field

A crop of field beans was planted in November 1947 in Great Field 1 at Rothamsted. It was on level ground with the fields to the north-east sloping gently down towards the main laboratories which are between $\frac{1}{4}$ and $\frac{1}{2}$ mile distant and about 60 ft. below the level of Great Field 1. All round in the remaining directions within about $\frac{1}{4}$ mile radius is flat and fairly open (Text-fig. 1). Trees and hedges were the main obstructions and these were either few or distant except along the south-west edge of the field where there was an avenue of large oak and lime trees. Along the south-east edge was a grass verge 18 ft. wide with a scanty hawthorn hedge 3–5 ft. high with many gaps and a few trees. The north-east edge was exposed along that half of its length adjacent to Great Field 2 (a pasture), while the other half was somewhat sheltered by a hedge 30 ft. away from the crop. The north-west edge was completely exposed along its whole length; the adjacent part of Great Field 1 was under barley which remained about as high as the beans during the primary migration and throughout the rest of the growing season; between the barley and beans was a strip of bare earth 15 ft. wide (Text-fig. 2).

Near the middle of the crop was an area of bare ground 100 yd. by 8 yd. Through the crop from north-west to south-east ran narrow paths $1\frac{1}{2}$–3 ft. wide at intervals of a few yards; these paths became somewhat obscured as the crop reached full height.

The crop itself was fairly uniform except in the north-west corner where there was a considerable area with small plants far apart. There were no large bushes of *Euonymus* or *Viburnum* within 1 mile; and winter hosts to the windward were relatively few and very scattered.



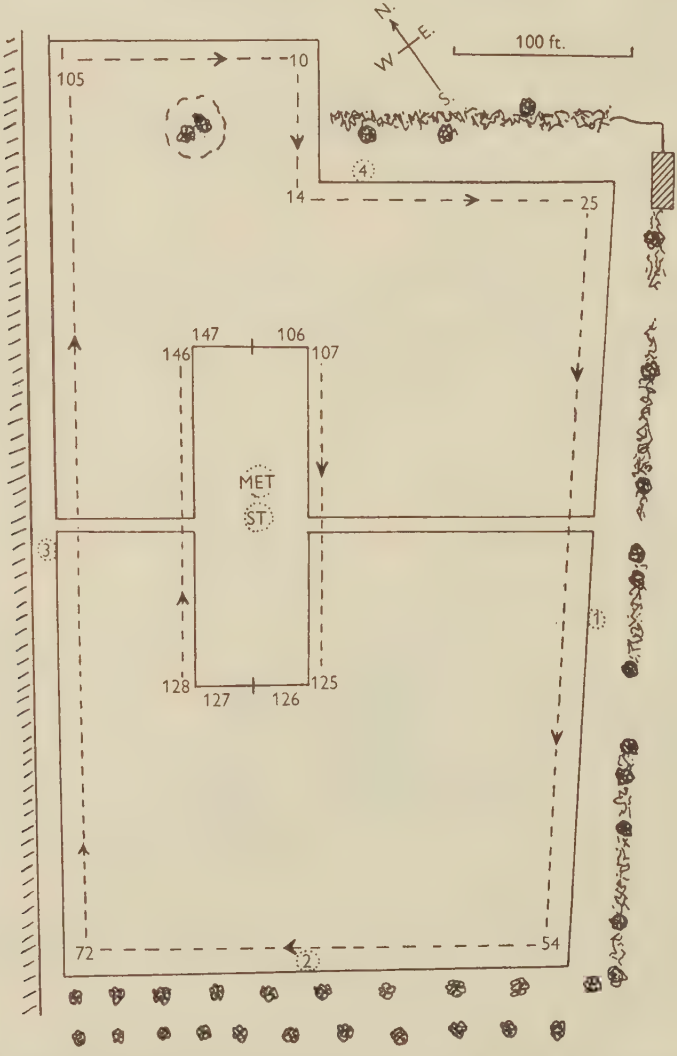
Text-fig. 1. General plan of Great Field 1 and surrounding fields—Bean plots are cross-hatched.

Method of assessing the infestation round the field

The perimeter of the field and the edges of the bare central area were pegged out into numbered 5 yd. sections. Two workers took each 5 yd. section and endeavoured to inspect every stem over an area extending 5 yd. into the crop. Thus a 5 yd. strip

all round the crop was inspected by the same two workers. Only those infestations extending down the stem from the crown for more than approximately 1½ in. were recorded; these were classified into two categories of approximately 1½–3 in. and over 3 in. long.

Many small infestations of 1 in. or less were not counted. These would grow and



Text-fig. 2. Plan of that part of Great Field 1 under beans in 1948. Numbers around edges refer to numbered 5 yd. sections. Positions of sticky traps 1 to 4 shown by dotted circles; *ST* = suction trap.

then be taken into consideration when a second count was made later. Two complete surveys of the crop edges were made in this way on 4–8 and 24–25 June respectively.

Tables 4 and 5 give the count of infested stems in each section for the two separate assessments. In Text-fig. 3 the mean numbers of infested stems per 5 yd. section for each side are indicated.

TABLE 4. *First assessment of Great Field I.* Aphis fabae *infestation 4–8 June 1948*

(A = nos. of colonies approx. 1½–3 in. long. B = nos. of colonies approx. over 3 in. long.)

Sides of field													Centre					
N.E.			S.E.			S.W.			N.W.				S.E.			N.W.		
Plot nos.	A	B	Plot nos.	A	B	Plot nos.	A	B	Plot nos.	A	B		Plot nos.	A	B	Plot nos.	A	B
1–5	59	2	26–30	26	0	55–59	3	0	73–77	29	1		106–110	58	1	127–131	45	7
6–10	16	0	31–35	18	0	60–64	11	0	78–82	59	5		111–115	13	2	132–136	13	2
11–15	30	1	36–40	31	1	65–69	3	0	83–87	11	0		116–120	44	1	137–141	7	0
16–20	33	0	41–45	34	1	70–72	4	0	88–92	44	1		121–126	87	8	142–147	90	2
21–25	24	0	46–50	12	0				93–97	27	0							
			51–54	7	0				98–102	34	3							
									103–105	27	1							
Totals	162	3		128	2		21	0		231	11			202	12		155	11
Mean no./5yd.	6·5	0·1		4·4	0·1		1·2	0		7·0	0·3			9·6	0·6		7·4	0·5

TABLE 5. *Second assessment of Great Field I.* Aphis fabae *infestation 24–25 June 1948*

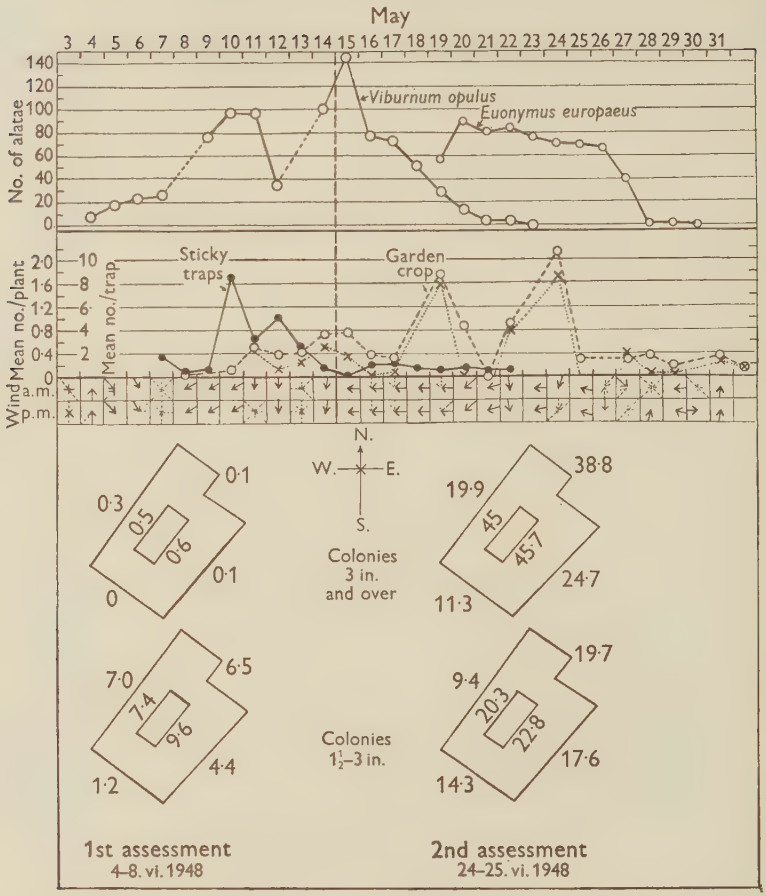
(A = nos. of colonies approx. 1½–3 in. long. B = nos. of colonies approx. over 3 in. long.)

Sides of field												Centre					
N.E.			S.E.			S.W.			N.W.			S.E.			N.W.		
Plot nos.	A	B	Plot nos.	A	B	Plot nos.	A	B	Plot nos.	A	B	Plot nos.	A	B	Plot nos.	A	B
1–5	82	319	26–30	73	165	55–59	56	37	73–77	46	131	106–110	121	142	127–131	133	283
6–10	65	132	31–35	105	145	60–64	111	62	78–82	98	129	111–115	87	144	132–136	79	186
11–15	100	238	36–40	74	146	65–69	48	40	83–87	79	112	116–120	110	232	137–141	94	112
16–20	177	169	41–45	80	110	70–72	43	64	88–92	50	87	121–126	163	442	142–147	120	363
21–25	68	112	46–50	85	96				93–97	9	12						
			51–54	94	54				98–102	9	43						
									103–105	27	142						
Totals	492	970		511	716		258	203		309	656		481	960		426	944
Mean no./5 yd.	19·7	38·8		17·6	24·7		14·3	11·3		9·4	19·9		22·8	45·7		20·3	45·0

THE EFFECT OF IRREGULARITIES IN THE CROP AND ASSOCIATED EDDY CURRENTS

Where narrow pathways came to the edge of the crop it was noticed that there was often an unusually heavy infestation with a stunting on either side of the pathway. Phosphorus was burned to produce a dense white smoke along the edges of the field, and when the wind was parallel to the edge of the crop the smoke from the phosphorus entered the pathways, at right-angles to the main wind direction, and travelled slowly along them for a considerable distance (Pl. 13, figs. 1, 2).

These eddies may be important in creating local infestations, but it is not known whether the aphids would fail to enter the crop if the openings were absent. The slow current of air between two rows of plants might also cause infestations to spread down the rows, a point often made with regard to the spread of virus in a potato crop.



Text-fig. 3. Composite diagram of wind direction, primary migration and infestation of bean field edges. Dotted graph beneath garden crop counts is a graph calculated on the assumption that 50% of aphids on the untouched half of the plot passed over to the hand-picked plants during the 24 hr. after hand-picking. Figures around the four plans of the field are mean numbers of infested stems per 5 yd. section.

Discussion and interpretation of results

Although the intensity of infestation on the different edges of the crop can be related to wind direction during primary migration, the picture is blurred. This was expected as the final infestation pattern is undoubtedly influenced by many inter-related factors such as local wind eddies during migration, the movements of migrants within the crop after their arrival, the effects of parasites and predators and

of local climatic influences during population growth. The situation is further complicated by the fact that aphid flight is at its maximum in calm weather when directive influences of wind would be at a minimum; and although fewer aphids fly on windy days a large number may, nevertheless, be blown into the crop on a windward edge. The integrated effect of these two opposing tendencies and the importance of the distance of primary hosts from the bean field have not been studied.

During the period of primary migration up to 14 May the wind was mainly from the north-north-west and north for nearly 5 days and mainly from the east-north-east for another 3 days. The largest colonies of 3 in. and over, which may be assumed to have developed from the first migrants, were most numerous on the north and north-east, or the windward, edges while the opposite sides of the field were almost free (Text-fig. 3). This result would be expected if the quantitative influx of migrants is highly dependent on wind direction. The same holds good for the colonies of $1\frac{1}{2}$–3 in. long both round the perimeter of the field and along the edges of the central area.

During the rest of the migration period after 14 May the wind was almost entirely from the east and north-east; this was associated with a reversal of the relative intensity of infestation from one side of the field to the other so that the heaviest infestations occurred finally on the north-east and south-east edges (Text-fig. 3). A population of *Aphis fabae* on sugar beet in a neighbouring field (Text-fig. 1) may have had an influence on the infestation of the north-east and south-east sides of the bean field even in calm periods. Observations on the crop in the nearby garden and on the winter hosts, however, suggest that relatively heavy migrations occurred after 14 May, and that the swing-over was likely to have been due to the wind direction apart from any other local influence.

Throughout the primary migration period the wind blew on to the north-east end of the field, and it is at this end that the heaviest infestation occurred. The opposite south-west edge, always on the lee side, had the lightest infestation. It could be argued, however, that the shelter given by the trees on this side may have contributed to this effect and had the trees been absent more infestation might have occurred there, particularly in calm periods.

The infestation pattern was, therefore, correlated with the wind direction during primary migration, but the data are insufficient to justify the conclusion that this pattern is directly due to the effect of wind direction.

In future work, plants along the crop edges should be sampled for incoming migrants during primary migration in addition to watching colonies on primary hosts; for this would give a direct measure of initial infestation and could be directly related to wind direction at the time.

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BROADBENT, L., DONCASTER, J. P., HULL, R. & WATSON, M. A. (1948). Equipment used for trapping and identifying aphids. *Proc. R. ent. Soc. Lond.* A, **23**, 57.

EXPLANATION OF PLATE 13

Figs. 1, 2. Wind parallel to the edge of the crop shown by phosphorus smoke to enter small pathways at right-angles to the wind direction. Infestation is particularly heavy on corners of the pathway entrance.

(*Received* 29 *December* 1949)

Fig. 1.

Fig. 2.

JOHNSON—*Infestation of a bean field by* Aphis fabae *Scop.*

METHODS FOR MASS REARING AND INVESTIGATING THE HOST RELATIONS OF *APHIS FABAE* SCOP.

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(With 7 Text-figures)

A method is described for producing some hundreds of apterous and thousands of alate virginoparous *Aphis fabae* every day continuously throughout the year. Broad beans are used as the host plants, in a 'production-line' arrangement of eleven ventilated breeding cages, with additional cages for starting subcultures and rearing alatae, all contained in a portion of a small greenhouse with supplementary lighting in winter. When running smoothly, the culture requires about half an hour's work by one man per day.

An 'aphid gun', being a modified type of aspirator catcher, is described, with which large numbers of aphids could be handled and counted rapidly without damaging them.

A modified technique for host-transference experiments is described, which permits individual leaves to be compared one with another as host-units for the aphids. Two types of small metal cages are used, both easily attached to leaves in their natural positions on growing plants. One type confines the aphids to a part of a single leaf and is used mainly for determining relative reproduction rates on different leaves. The other type encloses equal areas of two different leaves between which the aphids can move freely, and is used for determining their behavioural preferences. The principles underlying the use of such cages, the experimental procedures and the interpretation of the results obtained are discussed.

Introduction

The methods to be described were developed for work on various, mainly ecological, aspects of aphid host relations. Since the same methods were applied to several problems, and may be more widely applicable, they are given here separately and the results obtained by them will be described in subsequent papers.

A routine method for continuous mass production of *Aphis fabae*

Principles

The summer forms (apterous and alate *virginoparae alienicolae*) were chosen for routine production, owing to their capacity for indefinitely continued parthenogenetic reproduction. As far as was known the males and sexual females of autumn would always produce eggs, while the *fundatrices* and *fundatrigeniae* of early spring could be produced only from eggs, and the introduction of the dormant egg-stage as a regular part of the rearing routine was an undesirable complication. The routine production of winged gynoparous females would probably have been practicable, despite the

fact that their offspring lay eggs, because a proportion of virginoparous females continue to be produced by the virginoparous mothers under the laboratory conditions in which these mothers produce mainly gynoparae. The summer forms were, however, of greatest interest for the study of host alternation, because they do not normally infest the 'primary' host of the given aphid species.

Of the two summer forms, alatae were required for most of the experiments, and therefore in larger numbers than apterae. Maintenance of the stock, however, depended on the production of large numbers of apterae as well, because they settled more readily on a new host and were more fecund than the alatae. Thus the wingless form was used for the production of both wingless and winged forms. This was an advantage for experiments on winged forms, since the wingless form has a greater tendency to produce winged young than has the winged form under the same conditions (Shull, 1929).

Broad beans (*Vicia faba* L.) were chosen for routine breeding purposes, not only because this plant is probably the summer host to which *Aphis fabae* is best adapted, but also because it can be grown quickly and easily in large numbers. Each successive generation of aphids could be subcultured on fresh plants, which were used only during the days immediately following germination when they seemed to be at their most acceptable and nutritious for the aphids. Planted in clumps, the beans provided a compact, quickly growing mass of foliage and stems, able to meet the feeding demands of a large number of aphids for the short period necessary.

The final yield of aphids fell off when there were too many aphids in the initial infestation, because the plants became stunted and the aphids prematurely overcrowded, so that they began to wander off the plants, as well as to develop wings, before the maximum possible numbers had been produced. Experience showed that a satisfactory combination was fifteen beans in a 5 in. pot with an initial infestation of one hundred young, vigorous apterous adults. The adults of the next generation appeared in 4–5 days and began to bear young themselves. These adults were mainly apterous, having been born when the beans were at their most nutritious and the aphids were not crowded. The proportion of winged nymphs produced increased as the beans became older and the infestation denser, so that winged forms comprised the majority of the new adults appearing at about 1 week and became the overwhelming majority at 10 days. The winged forms left the plants and collected on the cage roofs, and many of the new apterous adults also began to leave the plants after 1 week, when they were collected for starting fresh cultures on new young bean clumps, or for experiment. By the tenth to eleventh day the population on the bean plants consisted almost exclusively of winged nymphs and even these were wandering off the nearly exhausted plants. Serious losses and malnutrition among the aphids were prevented by cutting off some of the most heavily infested bean shoots and laying them on new young bean clumps, to which the nymphs soon moved and where they were no longer overcrowded. As a result, large numbers of vigorous, winged adults were produced.

The varieties of broad bean used included Green Leviathan, Claudia Aquadulce and Seville Longpod. All proved satisfactory. John Innes pasteurized potting compost was used throughout, and three pots of fifteen beans were sown each day to provide a constant supply of newly germinated bean clumps.

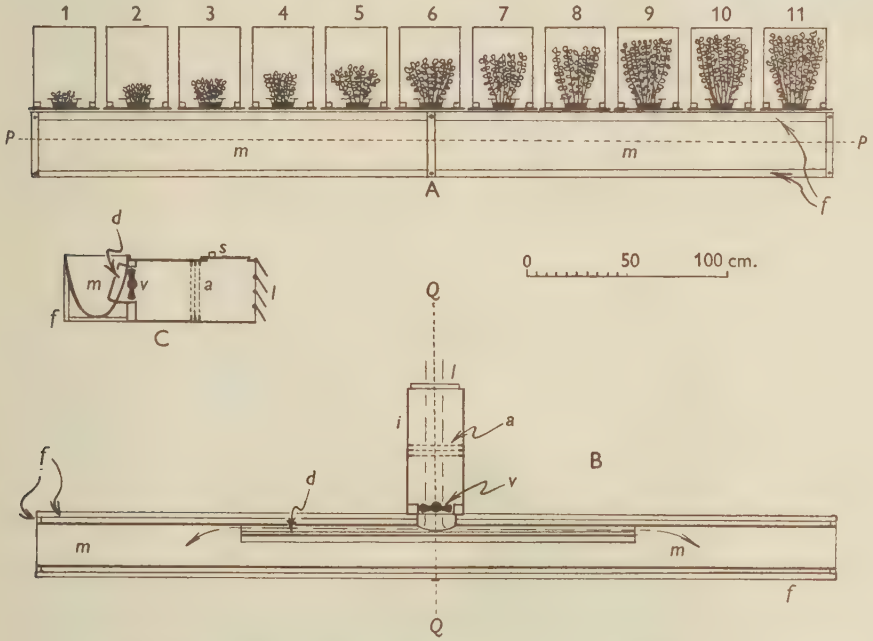

Fig. 1. Arrangement of main breeding cages and ventilation system. A, front elevation. B, section of ventilation system through *PP*, with air-flow shown by broken lines. C, vertical section of air ducts through *QQ*. 1–11, cages containing progressively older clumps of infested beans; *f*, angle-iron frame; *m*, main air duct; *d*, inner distributor duct; *i*, intake duct; *l*, louvred intake aperture in greenhouse wall; *s*, sliding door; *a*, air filters; *v*, 6 in. 'Ventaxia' fan.

Cages

There were three types of cage: one type for inoculating the new clumps, one for breeding and one for maturing the alatae. The *inoculation cages* were celluloid cylinders 10 cm. diameter and 8 cm. high, closed at the top with muslin. Placed over a newly germinated clump of beans, the cage served to confine the 100 young apterous adults to the immediate vicinity of the beans until they had settled down to feed. This they did in an hour or so, but the inoculation cage was usually left on until next morning when the pot with its infested beans was placed in a breeding cage.

The production-line arrangement of the eleven *main breeding cages* is shown in

Fig. 1, with details in Figs. 2 and 3. Each cage consisted of a lift-off cover and a 1·6 mm. ($\frac{1}{16}$ in.) thick aluminium base plate. Each cover was assembled on a framework of 0·5 mm. thick, 3 cm. wide, angle-aluminium, in six pieces: a base square, a roof square and the four corner uprights. These were held together by self-threading screws but were very light, the rigidity of the cage being due to the glass sheets forming the front, back and roof. The glass sheets were held in place by

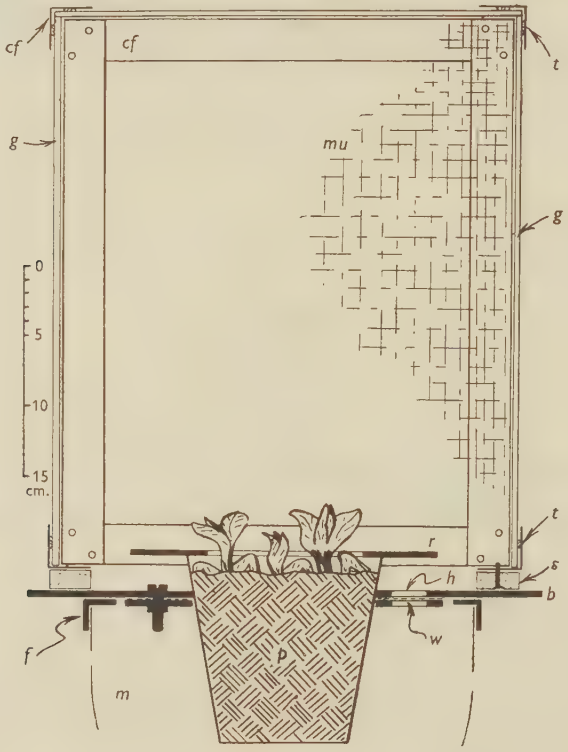

Fig. 2. Section of breeding cage in position on main duct. *f*, angle-iron frame; *m*, main air duct; *p*, 'whale-hide' pot with soil and beans; *b*, cage base-plate; *h*, air intake hole screened by wire gauze *w* supported by ring bolted to *b*; *r*, baffle ring; *cf*, angle-aluminium frame of cage cover; *gg*, glass sheets forming roof and front and back walls of cover; *mu*, muslin side wall; *s*, sponge rubber strip stapled to base of *cf*; *t*, soft twine.

the framework alone as will be seen in Figs. 3 and 3A. Final closure of the cages against the entry or egress of insects was effected by a number of separate components, including the 60-mesh, 44 B.s.w.g., phosphor-bronze wire gauze in the holes in the base plate, the sponge rubber strip stapled to the underside of the cover where it rested on the base plate, the glueing of the muslin walls to the inside of the frame, the soft twine packing between the metal frame and the glass sheets and the

use of so-called 'whale hide' pots. These pots were made of bitumenized paper, were soft enough to seal themselves into the central hole in the base plate and could be thrown away after use.

The bean clumps remained undisturbed in the breeding cages for a week or more, and a forced draught ventilation system was installed. Fresh air was drawn in from

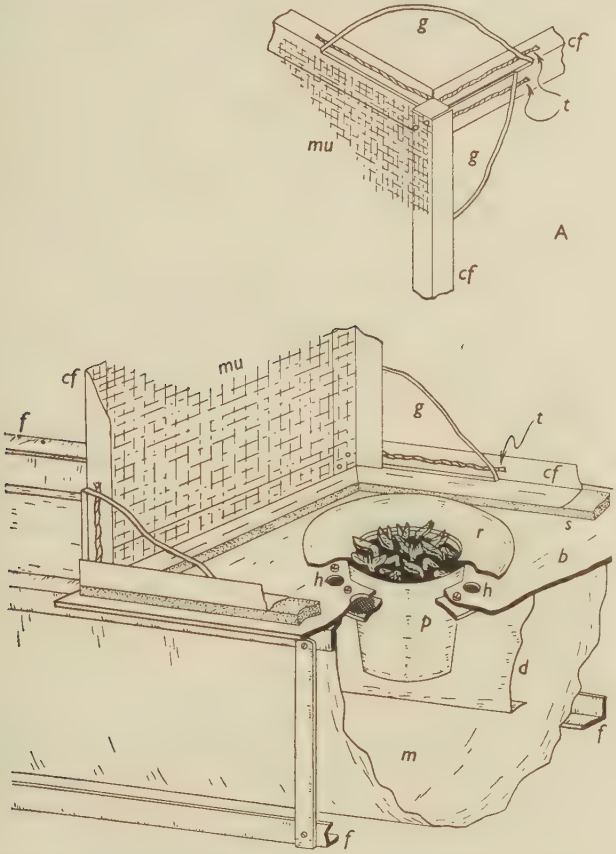

Fig. 3. Cut-away sketch of breeding cage in position on main air duct. A, detail of top corner of cage cover. *d*, inner distribution duct; lettering otherwise as for Fig. 2.

outside the greenhouse even in fairly cold weather for it warmed up in the ducting on the way to the cages, but in very cold weather warmer greenhouse air was taken in through the sliding door on the roof of the intake duct. With the eleven cages in position, their base plates formed a continuous roof for the main air duct (Figs. 1 C, 2 and 3) so that air driven into that duct by the fan could escape only up through the screened holes in the base plates, into the cages and out through their muslin side

walls. The flat baffle ring (Figs. 2 and 3) resting on the pot rim prevented the rain of honey-dew from the aphids from clogging the meshes of the air intake holes. At the same time it prevented the up-draught from these holes from playing directly on the aphids; otherwise the draught made them restless, although it was very gentle.

The main object of caging the bean clumps was to protect the aphids from their natural enemies. In 1947, when the aphids were less well protected, the stock had been nearly lost, once through the fungus *Empusa fresenii* Nowakowsky and once through the Braconid, *Aphidius* sp. The filters in the air intake duct were intended to reduce the intake of fungal spores, and the metal and glass cages were fairly easy to dismantle and sterilize if a fungal infection became established. The defences against fungal attack have not, however, been severely tested since these cages were in use, for the fungus epidemics among aphids in the Cambridge area in 1948 and 1949 were on a much smaller scale than in 1947. The various cage-sealing devices successfully excluded predators and ants. Parasites did occasionally become established in a cage, but it was not difficult to eliminate them from the culture when the cages were so well insulated one from another. Parasites did not pass from old to new colonies in the bodies of living aphids so long as only healthy-looking, actively wandering adults were selected to found the new colonies.

The base of the *alata-maturing cage* was a raised, sheet-metal platform with holes to take eight pots of beans. The lift-off cover was similar to the breeding cage covers, but had a stronger angle-aluminium framework, a 'Perspex' front, and muslin back, sides and roof. The young winged adults flew up to the light and gathered on the cage roof. Only one alata-maturing cage base was required, but there were three covers for it so that alatae of roughly known age could be collected by changing the cover at suitable intervals. The cage was not forcibly ventilated since its walls were largely muslin and its cover was often lifted.

Conditions

All three types of cage were kept in an ordinary small heated greenhouse. A diurnal temperature range of 18–25° C. was aimed at but not infrequently exceeded as the temperature-control arrangements were unsatisfactory. The main problem was to avoid over-heating in summer, leading to too rapid growth of both beans and aphid colonies and premature wandering of the aphids. Heavy shading and constant ventilation were not sufficient to prevent this in very hot weather. It is probable that the production line could be kept going under entirely artificial light which would allow much better control of conditions. As it was, temperature variations could be compensated for to some extent by increasing the number of apterae in the initial inoculation of each clump in the winter and decreasing it in the summer. Another measure to compensate for too rapid growth of the beans and the aphid colonies, was to terminate breeding on each clump at 10 or even 9 days instead of 11, thus omitting from use the last one or two cages in the line.

The variation of temperature was inconvenient, but did not affect the form of

aphids produced while long-day light conditions were maintained. Two 5 ft. 80 W. fluorescent tubes were installed end to end behind the cages. They were 18 cm. from the back walls of the cages, and gave about 2700 metre-candles in the middle of each cage. They were operated by time-switches to maintain a 16 hr. day throughout the year and continuous production of summer forms of the aphid resulted. When, owing to electricity cuts in the severe winter of 1946–7, both heating and lighting failed in the breeding house, the aphids developed *en masse* into gynoparae and the parthenogenetic stock was maintained with difficulty through the small minority of virginoparous apterae which continued to appear.

Procedure

The daily routine work on the mass culture may be summarized as follows:

(1) Remove and destroy the oldest clump in the alata-maturing cage.

(2) Remove the oldest clump (position 11) in the main production line, cut off the shoots laden with winged nymphs, lay them on a newly germinated clump and put it in the vacant place in the alata-maturing cage.

(3) Remove and wash the base plate, baffle ring and cover of the empty breeding cage in position 11. Slide all the other cages one place to the right and set the empty cage up again in the now vacant position 1. Re-seal the top of the main air duct by pushing the cage base-plates together.

(4) Collect 100 adult apterae found wandering in the cages in positions 6 to 10, drop them on to a newly germinated bean clump, cover with an inoculation cage and place in the empty cage at position 1.

(5) Remove the inoculation cage from the pot in the breeding cage at position 2 and set the baffle ring in place on this pot.

These operations could be completed by an experienced operator in $\frac{1}{2}$ hr. But when the component processes of the production cycle became 'out of phase' in some way, considerable time and trouble was needed to restore smooth running. The most common mistake was to leave colonies too long after they had reached their peak to avoid having to set up a new culture and in the hope of getting more aphids from the old clump. The net result of this was usually a loss, for more and more of the adults left the old clump and died on the cage floor and most of the winged nymphs that remained on it were too far advanced to benefit from their subsequent transfer to a new clump, and so developed into undersized adults.

Results

The culture maintained as above is a clone, which originated with a single apterous female taken from sugar beet in October 1946. It has been kept going parthenogenetically for some hundreds of generations until the time of writing, February 1950. When running well the system yields every day a few hundred adult apterae and a few thousand adult alatae.

HANDLING THE APHIDS

An improved method of handling large numbers of aphids was a necessity for the maintenance of the mass culture and more especially for the experiments. Except when transferring winged nymphs to the alata-maturing bean clumps, the aphids could not be moved *in situ* on cut-off bean shoots, since the adults were required separated from the young. Most of the adult alatae were not to be found on the

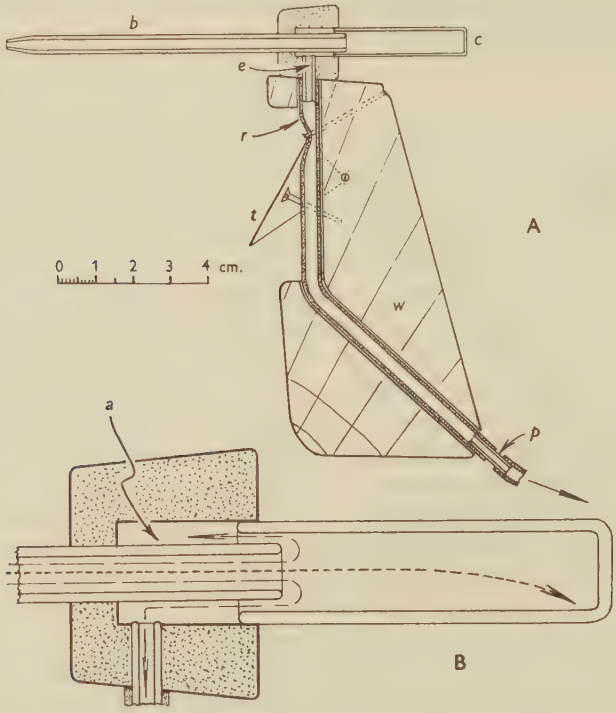

Fig. 4. The aphid suction 'gun'. A, section of complete gun. B, enlargement of rubber bung and attached parts, showing paths of movement of air (thin broken line) and aphids (heavy broken line) when trigger *t* is pressed. *b*, glass barrel; *c*, detachable glass collecting tube; *e*, glass air-exit tube leading from annular space *a* into rubber tube *r* which passes through tunnel in wooden butt *w* to glass connecting piece *p*.

bean shoots for they flew off and gathered on the cage roof. The aphids were required in counted lots in small glass tubes for loading the experimental cages. A glass suction pipette, with the tip drawn out and bent down, and the orifice carefully squared-off so as to make a good seal on a flat part of the insect's body, proved much better than a brush for handling individual insects, but even this method was too laborious for large numbers.

The requirement was met by incorporating the standard small holding tubes in an aphid 'gun'. This was a modification of the conventional type of insect aspirator, designed for very rapid action, to eliminate knocking about of the aphids and to facilitate counting during catching. Details of the aphid gun and its action are shown in Fig. 4. A similar system of air and aphid movement can be used with mouth suction, but this was tiring, and a vacuum pump with a large reservoir maintained at pressures between 25 and 35 mm. of mercury was a great advantage. The treatment suffered by the aphids drawn up the barrel by the inrush of air, could be controlled by the setting of the trigger stop-screw, by the speed of trigger-pressing and by the time the trigger was held depressed. With practice, the aphids could be drawn up and projected into the mouth of the collecting tube fast enough not to follow the air forwards and become trapped in the crevice between the barrel and the collecting tube, and yet not so fast as to impact on the end of the collecting tube. The aphids then landed gently on the cushion of dead air near the end of the tube, without having been dragged round any corners on the way. They could be counted in the collecting tube by a mere shift of the eyes during catching. When enough were in the tube it was removed, corked and replaced by an empty one. Given a cage cover with plenty of aphids on its walls, the attaching, filling with ten to fifteen aphids, detaching and corking of a tube took 15–20 sec.

Experiments on host relations

Principles

The methods to be described were intended to be used in experiments to elucidate the broad biological relations between the aphids and their plant hosts. The methods were not designed for the analysis of the stimuli provided by the plant to the aphid, of the reactions of the aphid to these stimuli or of the nutritional relations between the two organisms. Intact growing plants were therefore to be used and the experiments were, in principle, conventional 'host-transference' experiments. The usual procedure is to place a few aphids on each of a number of different plants and record the development or otherwise of colonies. A more natural method has been to set out the test plants in a ring round an infested donor plant and record the appearance of migrants and the subsequent development of colonies on the test plants (Jones, 1942*a*, *b*). But, for present purposes, it was desired to carry the analysis of events in nature one stage further, by using the individual leaf, rather than the whole plant, as the host-unit for study.

The whole plant is the host-unit for the agriculturalist, since the yield of a plant depends on the physiological co-operation of all its parts; and also for the virus student, since virus diseases are systemic. The whole plant is the host-unit used also by the aphid taxonomist and ecologist. Since aphids are morphologically very uniform as a group, yet polymorphic within the species, the aphid taxonomist has often to be an ecologist and experimentalist as well, employing the host relations of

the insects as an aid to their identification and classification. For these reasons, perhaps, aphid host relations have been studied mainly in terms of whole plants, even by ecologists (e.g. van der Plank, 1947, 1948; Broadbent, 1948). The relations between aphids and the different parts of plants have been much less systematically explored.

A plant of a given species is unequally susceptible even to the aphid species best adapted to it, not only at different stages of its development but also in its different parts at any one stage. This fact is made use of in practice; for example when the susceptible growing tips are pinched out from broad beans to protect them against black-fly. It is often mentioned in the literature (e.g. Doncaster & Gregory, 1948; Broadbent, 1948), but usually only in passing. Yet it seems a most promising starting-point for any causal analysis of aphid host relations, for the different and changing leaves of plants exposed to aphid attack, constitute an array of 'natural experiments' (Kennedy, Ibbotson & Booth, 1950; Ibbotson & Kennedy, 1950). If these could be systematically explored, then some broad principles concerning aphid host relations might be established. Until such principles are established, the search for deeper causes of a physiological, chemical or physical nature (Evans, 1938; Emery, 1946) necessarily remains somewhat arbitrary, and any findings are difficult to interpret.

The immediate object, however, of devising methods for the separate study of individual leaves as host-units for the aphids was ecological. The development and physiological activity of the whole plant is reflected in the visible pattern of growth, senescence, turgor, etc., running through its whole complement of leaves. If the relative susceptibility to aphids of leaves differing in such gross biological respects, could be determined, then an estimate might be made of the susceptibility of the whole plant, for comparison with other plants of the same or even of different species. Some light might thus be thrown on the causes of the changes in distribution of aphids among their available hosts, including the regular seasonal changes known as host alternation. What was required, therefore, was a means of comparing individual leaves as host-units for the aphids.

Since the immediate intention was to establish the relative attractiveness of different leaves in terms of their gross properties of growth, senescence, etc., it was not desired to exclude any reactions that might be important in the natural processes by which aphids discriminate between the plants and leaves available to them. The olfactometer method (Jones, 1943, 1944) or artificial feeding methods (Hamilton, 1935) adapted for preference measurement, did not meet this requirement. It was necessary to give the aphids direct access to intact leaves.

Casual observation suggested that, when one leaf acquired a heavier population of aphids than others, this was due both to more wandering aphids settling down to feed on that leaf than on the others, and to more young being produced by the aphids once they had settled down to feed there. Means of measuring both settling preferences and comparative reproduction rates were therefore required.

Leaf cages

Various types of cage have been described for confining aphids or other small insects on small areas of leaves on growing plants (e.g. Storey, 1928) but none seemed to meet the present requirements.

A type of cage was required which:

(1) Enclosed a standard area of leaf surface.

(2) Left the leaf exposed as freely as possible to light and air.

(3) Allowed easy examination of and access to the contained insects without disturbing them.

(4) Could be quickly and easily attached to a leaf in its natural position on a growing plant.

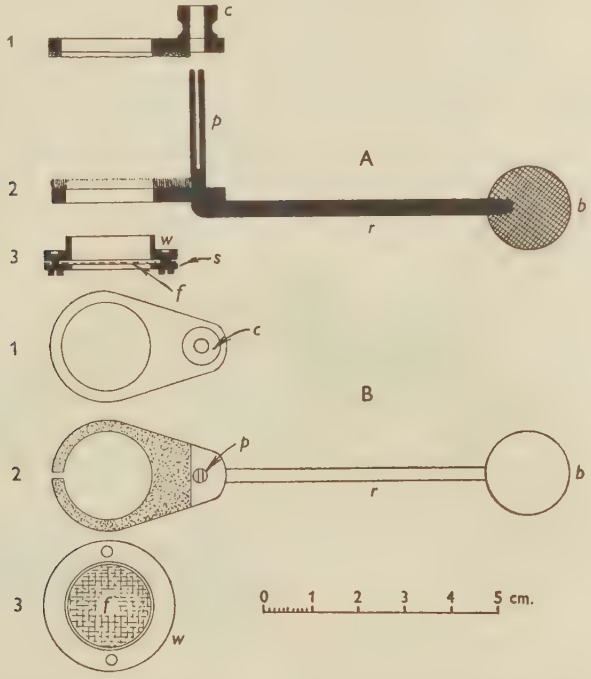

Fig. 5. The reproduction cage, separated into its three main components. A, section. B, plan. 1, leaf-clamping ring with milled collar *c*; 2, main piece with split-pin *p* and supporting rod *r* ending in plastic ball *b*; 3, aphid container made up of flanged wall-ring *w*, bolting silk floor *f* and floor-supporting ring *s* fastened to *w* by screws.

Two types of suitable cage were finally devised: one to take a single leaf and designed to measure mainly the relative reproduction rate of aphids on different leaves; and the other to take two leaves side by side and designed to measure the preferences of aphids for feeding on different leaves.

The single-leaf, or reproduction cage (Figs. 5, 7) was used to confine aphids to the under-surface of a portion of one leaf for several days. It consisted essentially of three brass rings one on top of another: an uppermost, or leaf-clamping ring, a middle or main-piece ring attached to a support and a lowermost or container ring.

The apposed surfaces of upper and middle rings were faced with lint and velvet, respectively, to avoid bruising the leaf and prevent even the smallest nymphs from escaping through crevices alongside veins on the leaf. The milled collar of the leaf-clamping ring slid up and down on the split-pin of the main-piece, so that the space between the rings could be adjusted to grip a leaf of any thickness without undue pressure. The clamping caused some distortion of rapidly growing leaves but did not otherwise leave visible traces on leaf areas caged for a few days.

The flange on the container ring fitted inside the main-piece ring to form the wall of the chamber containing the aphids. Owing to the break on its outer side the main-piece ring consisted in reality of two semicircular, slightly springy arms which gripped the flange of the container ring firmly but lightly enough for easy insertion and removal with two fingers. The bolting silk forming the container floor was dyed black to reflect the minimum of light. With the cage in position on a leaf the aphids could be examined through the bolting silk and silhouetted against the light coming through the leaf. The use of a small dental mirror obviated the necessity of bending to look up into the cage from below.

The plastic ball at the free end of the supporting rod fitted into an adjustable socket at the end of a 40 cm. length of electric cable, the other end of which was encased in a metal sleeve which could be clamped to a suitable fixed rod set up near the plant. The adjustable clamp on the sleeved end of the cable and the flexibility of the cable itself provided a two-stage coarse adjustment, and the ball-and-socket joint a third, fine adjustment, in the process of fitting the cage to a leaf (Fig. 7). With this form of supporting arm, any chosen leaf could be caged in a few moments in its natural position on a growing plant.

The double-leaf, or preference cage (Figs. 6, 7) was used to confine aphids to the under-surfaces of two equal areas of different leaves held side by side, the insects being free to move from one to the other and settle down on whichever they preferred.

The leaf-clamping piece, made of plastic for lightness, was held down on the leaves by the upper cover-plate, with its milled collar which slid down the split-pin fixed in and projecting upwards from the main-plate. Lint and velvet facings again served to prevent bruising of the leaf and the escape of small nymphs.

The oval piece of the aphid-container formed the walls of the chamber, floored with bolting silk, in which the aphids were free to move from one leaf window to the other. The container was held against the mainplate during use by the lower cover-plate with its milled collar sliding on to the split-pin projecting downwards from the main-plate. The flange along one edge of the main-plate served to prevent rotation of the container on the main-plate.

The plastic parts were painted black, the upper and lower cover-plates were faced on the inside with baize, and the container was faced with lint where it pressed against the main-plate, in order to exclude light from the cage interior after the aphids had been inserted. If the cage was not darkened in this way, the newly

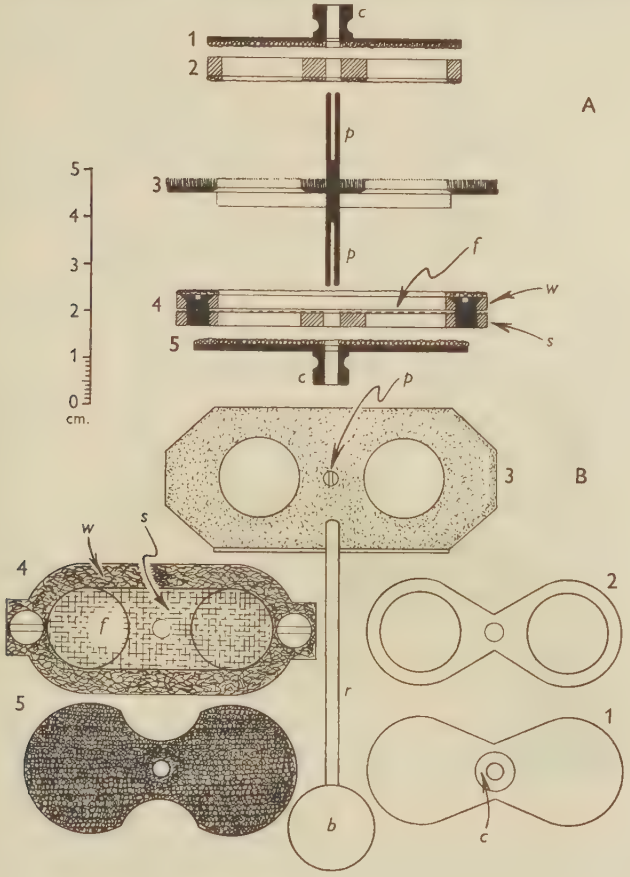

Fig. 6. The preference cage, separated into its five main components. A, section. B, plan. 1 and 5, upper and lower metal cover-plates, with milled collars *cc*; 2, plastic leaf-clamping piece; 3, metal main-plate, with upper and lower split-pins *pp* and supporting rod *r* ending in plastic ball *b*; 4, aphid container, made up of oval plastic wall-piece *w*, bolting silk floor *f* and plastic floor-supporting piece *s* fastened to *w* by screws.

loaded aphids sometimes moved toward the better-lit end of the cage before settling down on a leaf, which interfered with the display of their feeding preferences. It was less important to keep the leaf surfaces open to light and air during these experiments than during the reproduction experiments, for the standard preference experiments

lasted only 1 or 2 hr. after which the leaves could be exposed by rotating the cover-plates into the transverse position (as in Fig. 7), or the leaves could be released from the cage. During an experiment the aphids could be counted on the two leaf windows by momentarily swivelling the cover-plates into the transverse position and looking up at the windows from below, through the bolting silk, with the aid of a mirror. The preference cages were supported on arms exactly like those of the reproduction cages.

Fig. 7. Sketch of leaf cages in position on spindle and beet leaves. *RR*, reproduction cages seen 1, from above and 2, from below; *PP*, preference cages seen 1, from above and 2, from below. *S*, stand to which are clamped *aa*, cage-supporting arms ending in *bb*, ball-and-socket joints.

Procedure

Having attached the cages to the chosen leaves and collected the requisite number of small tubes containing aphids, the cages were loaded. As a rule, ten parent aphids were loaded into each reproduction cage and thirty into each preference cage. This was done by tapping the tube until the aphids fell to the bottom, removing the cork, tipping the aphids out into the detached container of a cage and immediately re-attaching the container to its cage. In order to ensure an equal initial

distribution of the aphids loaded into the preference cages, fifteen aphids were tipped simultaneously from each of two tubes into each half of the container.

In comparative reproduction experiments, readings were taken of the number of parents alive, the number settled on the leaf and the number of young produced, at appropriate intervals after loading, until thirty or more young were present in some cages, a matter of days in most of the outdoor experiments. In the preference experiments, four readings at 20–30 min. intervals were usually taken of the number of adults settled on each of the two leaves. The cages were then opened, emptied and re-loaded with fresh aphids for a second set of readings. It was found that aphids which were observed to remain stationary on a leaf for the few moments of counting, nearly always had their mouthparts inserted into it, presumably feeding.

Conditions affecting the results

As the experiments were done on growing plants either in a greenhouse or outdoors, a major influence on the behaviour of the aphids was the weather, especially the amount of sun. The activity of the aphids varied inevitably over a wide range, which meant that the results obtained with different types of leaves could be compared only after simultaneous loadings, within one experiment. This difficulty was met by using several sets of cages in each experiment: one set of reproduction cages for each leaf type, or one set of preference cages for each pair of types. This was made possible by the rapidity with which a number of cages could be set up and loaded.

The variations in activity level created another kind of difficulty in the preference experiments, although not in the reproduction experiments. If the aphids were inactive in the reproduction cages and remained settled on the leaves, this was an advantage, for more young were then produced and a clearer measure was obtained of the relative reproduction rates, uncomplicated by differences in the time the aphids spent wandering round the cages instead of feeding. If the aphids became very active in the reproduction cages their death-rate rose and reproduction rate fell, but the small numbers of young produced retained some comparative value between one leaf type and another.

In the preference experiments, however, changes in activity level had a direct effect on the comparative value of the counts of aphids settled on different leaves. There was a certain optimal activity level at which the aphids' powers of discrimination were most clearly expressed in their unequal distribution between the two leaves in a cage. If their activity rose above or fell below this optimal level the apparent strength of their preference for one leaf was reduced. This is a difficulty encountered in all work concerned with the distribution of a number of animals between two freely communicating, contrasted zones, such as the two halves of an 'alternative chamber' (cf. Kennedy, 1947). When the aphids were very inactive they settled on the first leaf they met in the cage and stayed there for the 2 hr. of the experiment. As a result they were distributed at random between the two leaves, and an unequal distribution

did not necessarily mean they had exercised any preference. When the aphids were very active they stayed feeding for so short a period in either leaf that the total number found feeding at any one reading was small, and again their distribution became more random.

These difficulties, due to variations in the activity level of the aphids, could be overcome to some extent by deliberately varying other conditions affecting their activity, primarily the condition of the aphids themselves as determined by their *previous* activity. Broadbent (1949*a*) has shown that the activity of winged *Myzus persicae* decreases with age, starvation and previous activity. The aphids were denied access to plants during his activity tests but they suggest that young, well-fed aphids will be less inclined to settle down and feed on a plant than older, exercised or starved ones. Ibbotson (unpublished) has shown an analogous increase in the frequency of probes made 'spontaneously' by *Aphis fabae* on inert surfaces, as a result of starvation. These findings were made use of, and roughly confirmed, in the preference experiments, by pre-treating the aphids in the appropriate way when the weather was such that too great or too little activity was to be expected in the cages. Leaving the alatae in the maturing-cage cover, empty of plants, in bright, warm conditions where they were very active, reduced their subsequent activity in the preference cages and so compensated for the immediately activating effect of bright, warm weather. Using fresh alatae which had recently flown off the plants and had been kept in cooler, darker conditions before loading, helped to keep up their subsequent activity in the cages despite immediately inactivating, dull, cool weather.

Such procedures might not be justified if Moericke's (1941) views on the effect of age on activity were correct. He divides the life of the winged aphid into two phases: an earlier migratory phase ('Zugphase') and a later settling phase ('Befallsphase'). Flight takes place, on this view, in both phases, but in the first phase it is a swarming or emigration flight ('Schwärmflug') and in the second it is something fundamentally different, host-finding flight ('Siedlungsflüge'). If this were true, then it might be expected that the aphids' tendency to discriminate between leaves of different types would be weak or non-existent when the aphids were young, and would develop only in the 'Befallsphase'.

However, Moericke does not give adequate evidence to justify these sharp distinctions, and we cannot confirm their existence. A winged adult will settle down to feed for days together, whatever its age, if the plant and other conditions are suitable. Thus we have transferred newly moulted winged *A. fabae* from the plant upon which they became adult to sprouting beans, and *Myzus persicae* to sprouting potatoes, by means of a camel-hair brush. There they remained, feeding and bearing young for days, if cool, moist, dull conditions prevailed. It seems that, although Broadbent (1949*a*) found that aphids are more lively at 1–4 than at 4–8 days old, they will not refuse to settle at any age, and that other conditions play a more important part in determining whether they feed or fly. Given the right conditions, we have found day-old or even younger adults showing strong preferences in leaf-cages,

and know of no evidence that older ones are more discriminating: they may well be less so.

Handling the preference results

The activity level of the aphids affected the extent to which unequal distributions recorded in the preference cages were due to active discrimination or were merely accidental. In collating a number of results obtained at different times, therefore, little meaning could be attached to differences in the apparent strength of preferences, that is in the size of the majority of aphids recorded on one of two leaf types caged together. It was deemed more useful to divide the results of a series of loadings of a set of cages containing similar pairs of leaf types, into three classes: (i) cases where the aphids were found settled in a significantly larger proportion on one of the types; (ii) cases where a significantly larger proportion was found on the other type; and (iii) cases where the proportions on the two leaves were insignificantly different.

The larger the total number of aphids that settled, the more significance could be attached to any disproportion between the numbers counted on the two leaves, as indicating a real preference. The total number of settled aphids had therefore to be taken into account in deciding whether a given majority on one leaf could be classed as significant or not. This was done in the following way: the mean number of aphids counted on each of the two leaves, in the course of the four readings after each loading, was first worked out. The difference between these single-leaf means was then compared with the square root of the mean number of aphids counted on the two leaves combined. If the difference between the means for the two separate leaves was greater than the square root of the mean total on both leaves, the majority was classed as significant. When that occurred the result was called, for brevity, a *win* for the leaf with the majority of aphids on it. If the difference between the separate leaf means was smaller than the square root of the combined mean, the difference was classed as insignificant and the result was called a *draw*.

It will be seen that the chosen level of significance was low. In any one case a 'significant' majority or *win* as adjudged in this way might not infrequently be a matter of chance rather than evidence of a real preference on the part of the aphids. The method was not designed for single cases but for a series of comparable cases. So long as the chosen level of significance was the same for all cases, as the method ensured, the existence of a real preference for one of two leaf types could be judged by the proportion of *wins* gained by that leaf type among the results of the whole series of loadings. The individual significance of each result was immaterial since chance *wins* should be distributed equally between the two leaf types in a series of results.

Inspection of a large body of results showed that preferences were frequently expressed in majorities that were small, but gained repeatedly by one rather than the other of two leaf types. A low level of significance was therefore chosen deliberately as the standard for individual results. The main object of these experi-

ments was to establish merely the existence and direction of preferences, not to determine how strong the preferences were in the cages, for in nature their effective strength would depend on many variables which the experimental method was designed to exclude.

Interpretation of results

Use of the preference cages showed that the aphids did indeed discriminate actively between leaves of different types, and permitted the pattern of their preferences to be worked out. But it is not known by exactly what reactions to what stimuli aphids exercised their preferences for feeding on one leaf rather than another. Sight was excluded in the two-leaf cages because the aphids were there in the dark, but previous (unpublished) work had suggested that aphids do not select their host-plants by sight. Reactions to chemical or physical stimuli acting on the aphids before they have penetrated the leaf surface, were not excluded in the cages and may well have played some part in causing the aphids to settle on one leaf rather than another. Such reactions are commonly assumed to exist and even to come into play at a considerable distance from the plant (e.g. Profft, 1939; Doncaster & Gregory, 1948), although the evidence of such directed reactions is meagre (Jones, 1943, 1944; Heinze, 1949; Broadbent, 1949*b*).

It is known, however, that reactions to stimuli received before insertion are not decisive for these aphids as a means of discriminating between different leaves. *Aphis fabae* and *Myzus persicae* have often been seen to insert their mouthparts into leaves on which they will not stay and feed for long and do not colonize. They also probe readily on surfaces such as glass or wax in the laboratory. As to the form of their reactions, it was mentioned that aphids loaded into preference cages under conditions which discouraged wandering activity, inserted in the first leaf they met and remained there, randomly distributed between two leaves for one of which, when conditions encouraged wandering, they showed a marked preference. The profound effect that changes in the aphids' activity level had on the strength of the preferences they showed, suggests that their discrimination was itself mainly a matter of differential activity. That is to say their preferences seemed to be exercised through *kineses* rather than *taxes*: by moving less when on one leaf than on the other, rather than by moving preferentially *toward* the one leaf. The decisive element in discrimination between leaves seemed to be the time the aphids remained to feed after inserting their mouth parts.

In practice, the possibility of discrimination before insertion, whether directed or undirected, was probably somewhat diminished in the cages by comparison with the situation in nature. By caging the aphids against leaves their purely chance encounters with a leaf surface, and with other aphids already feeding, were greatly increased, and encounters of both kinds elicit probing (Ibbotson & Kennedy, 1950). Also, completely closing the preference cages during an experiment would render gradients of odour-diffusion less sharp than in the open.

Thus it seemed probable that the preferences recorded in these experiments were, as they probably are also in nature, due mainly to reactions to chemical or physical stimuli from the interior of the leaves. If so, for provisional working purposes, the preferences may be described as primarily *feeding* preferences.

Use of the reproduction cages showed that the aphids reproduced at different rates on different leaves, but did not give unequivocal evidence of a purely nutritional component in their unequal fecundity, for the behavioural component could not be eliminated even in cages containing only one leaf. Some indication of the independent role of nutritional differences could be obtained, but this matter is best left for further consideration in the light of actual results (Kennedy & Booth, in press).

The main point to be allowed for in applying the results obtained in these experiments to events in nature, is that the aphids were caged. If they wandered off the leaf surface they were soon brought back to it by the cage walls. If aphids become more inclined to settle and feed as they become older or more starved (p. 466), then they will settle eventually on a relatively unfavourable leaf in a small cage, although in nature they would often be able to leave it, find a more favourable leaf, or die. Hence the effective reproduction rates of batches of aphids offered different leaves, probably differed less in the cages than they would between similar leaves in the open. Similarly, the proximity and small size of the two areas of leaf offered side by side in the preference cages, meant that aphids moving only a small distance on the preferred leaf found themselves at its edge, wandered off and often encountered the other leaf and settled for some time on it before they came upon the preferred leaf again. The facility of moving from one leaf to the other, combined with the limited time allowed for the aphids to accumulate on one leaf, and with their gregarious tendencies (Ibbotson & Kennedy, in press), made their preferences seem much weaker than they do in nature. A similar effect seemed sometimes to be produced through the action of shading and enclosure on the leaves themselves, even for the short experimental period, as will be seen in a later paper.

Thus the methods described give results further removed from events in nature than do host transference experiments of the conventional type. The value of these methods is that they show systematic differences between leaves as well as between plants, in a conveniently replicable way. The differences shown may often be small, but much larger difficulties of the same kind are observed among free aphids and whole plants (Kennedy *et al.* 1950; Ibbotson & Kennedy, in press), so that the principles revealed in the cage experiments may be assumed to play a larger and more important part in the aphids' host relations in nature.

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THE TOXICITY OF CERTAIN SYNTHETIC ORGANIC COMPOUNDS TO THE FRUIT-TREE RED-SPIDER MITE

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The acaricidal activity of ninety synthetic organic compounds has been investigated in the laboratory. Considerable differences were found between the winter eggs, the summer eggs and the adult female stages of the fruit-tree red-spider mite (*Metatetranychus ulmi* (Koch)) in their susceptibility to many of the compounds but two substances, namely azoxybenzene and *n*-dodecyl thiocyanate, were appreciably toxic to all three stages. Of the compounds showing promise against summer eggs and mites, bis-(*p*-chlorophenyl) methyl carbinol and 4-chloroazobenzene were the best, being highly toxic to both summer stages. Of the remainder, diphenylsulphone, 4-chlorodiphenylsulphone, azobenzene and hydrazobenzene were highly ovicidal, while C_{12}–C_{13} alkyl thiocyanates and *sec.*-dodecyl thiocyanate gave high kills of the adult female mite.

Relationships between molecular structure and acaricidal activity have been sought. In a series of compounds characterized by the presence of two benzene nuclei connected by certain bridging groups, it has been found that activity to the summer stages of the mite can be influenced by alterations to the bridging group and by substitution in the benzene nuclei. Maximum activity appears to be associated with chlorine substitution in the *para* position in one nucleus and also with compounds of this type having unsubstituted nuclei.

Introduction

In recent years the fruit-tree red-spider mite, *Metatetranychus ulmi* (Koch) (= *Paratetranychus pilosus* (C. & F.)), and related Tetranychid mites have become pests of considerable economic significance in many parts of the world. This increase in importance is largely due to the widespread use of insecticidal washes which have had little or no effect upon the mites but which have seriously reduced the numbers of insect predators which previously kept the mites under control. Tar-oil winter washes were the first insecticides to show this effect with *M. ulmi* (Massee & Steer, 1929), and in addition they destroyed the moss and lichens on the trunks and branches which had formerly been the habitat of overwintering predaceous insects, thus further favouring the advance of the mite. The more recent use of the chlorinated hydrocarbon insecticides, D.D.T. and benzene hexachloride, has accelerated the increase in the red-spider populations due to this preferential destruction of the predators, and it is clear that active measures must be taken to deal with the pest if it is to be kept under control. The fruit-tree red-spider mite overwinters on the tree in the egg stage, and when these eggs hatch in early summer

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a period at first occurs during which a maximum proportion of mites exists on the leaf and a minimum proportion of summer eggs are present. Later, during the summer, the red-spider population includes summer eggs, immature stages and adults all present together on the leaves of the infested trees. It will be seen that control measures can be directed at the winter egg in the dormant season, at the first generation of mites in early summer, or later at the complex of summer stages. The current commercial practice is to apply D.N.C. winter washes containing at least 3 % of petroleum oil to kill the winter eggs, followed by a summer application of derris insecticide or summer petroleum, carefully timed to occur when most winter eggs have hatched and only a few summer eggs have been laid. This summer treatment is necessitated (Austin & Massee, 1947) by the speed with which large populations of the mite build up under favourable conditions from the small percentage of winter eggs which escape destruction; unless these summer control measures are effective, undersized fruits and severe bronzing of the foliage may occur, followed by premature defoliation.

There are disadvantages in the control measures at present adopted. As the D.N.C.-petroleum winter wash may cause bud damage, a non-phytotoxic chemical for winter control is needed. Again, in the summer treatments the risk of foliage damage with the petroleum oil is a disadvantage, especially when applied within a few days of a lime-sulphur treatment. Derris is not highly toxic to the summer eggs and hence needs accurate timing of application.

Against the winter eggs only *n*-dodecyl thiocyanate (Bennett, Kearns & Martin, 1947) has so far been shown to be appreciably toxic, and even with this acaricide the rather high concentration of 0·4 % was necessary to give kills approaching 100 %. More progress has been made in the search for synthetic toxicants to the summer stages. Kagy & McCall (1941) showed that the di*cyclo*hexylamine salt of 2:4-dinitro-6-*cyclo*hexylphenol was very toxic to this mite, and this has since been confirmed by many workers including Simpson (1945), Cakebread (1947) and Ross (1947). Unfortunately, this material has phytotoxic properties and cannot be used with alkaline sprays such as lime sulphur owing to the liberation of the free alkali phenate which makes it even more dangerous to foliage. However, work on selecting safer formulations of 2:4-dinitro-6-*cyclo*hexylphenol has been in progress for some years, and in 1947 Ross was able to recommend tentatively the ammonium salt of the phenol for trial by growers in Ontario. Hey (1946), following up earlier work by Blauvelt (1945) on other Tetranychid mites, investigated the effectiveness of azobenzene as a wet spray on apple trees. He reported that at 0·1 % it gave complete kill of all active stages and 50 % kill of summer eggs but caused some defoliation.

Armstrong (1947) using a glasshouse technique found that bis-(*p*-chlorophenyl)-methyl carbinol (D.M.C.) was extremely toxic to the active stages of the mite giving 96–100 % kill at a concentration of 0·025 % active material; it was also outstanding as an ovicide. D.M.C. had good residual action, was non-phytotoxic and was compatible with all the commonly used spray materials, but large-scale

field confirmation of these latter points is still desirable. Another substance, namely bis-(*p*-chlorophenoxy)methane (D.C.P.M.), was also stated to give almost 100 % kill of mites at 0·1 % concentration. Two organo-phosphorus compounds were examined, *p*-nitrophenyl-diethyl thionphosphate (Parathion) and hexaethyltetraphosphate (H.E.T.P.). Parathion at 0·015 % destroyed 98–100 % of all active forms, whereas H.E.T.P. at 0·0625 % gave 90 % kill of the active stages. No attempt was made by Armstrong to assess the ovicidal properties of the substances tested, but occasional notes on egg hatch are given. Reports have already been published by Eaton & Davies (1948, 1950) and Eaton (1948, 1950) giving preliminary results on the toxicity of organic compounds to the fruit-tree red-spider mite. Some of the data on which these summaries were based are included in the present work, together with results not hitherto reported. The main object of the paper is to present laboratory data which would facilitate the choice of materials for large-scale field spraying trials for the control of the mite, but an attempt has also been made to indicate some relations between toxicity and chemical constitution, in the hope of achieving a more fundamental understanding of acaricidal activity.

Experimental

In investigating the toxicity of synthetic organic substances to the fruit-tree red-spider mite, it is necessary to consider the susceptibility of three groups of stages—the winter egg, the summer egg and the post-embryonic summer stages of the mite. Ideally it would have been desirable to examine the susceptibility of all the summer developmental stages (larvae, nymphs, adult males and females) to the potential acaricides, since, by analogy with other pests, one would expect the different stages to differ in their resistance, but their small size and activity makes this rather difficult. Consequently, in addition to winter and summer eggs, tests were made on adult females which, being largest and rather less active, are easiest to count. The biological testing techniques adopted were those of Eaton & Davies (1950). In Tables 1–7 all figures for percentage kill have been obtained by correction for the mortality occurring in the control experiments where no toxicants were used in the dipping liquids (Abbot, 1925). On any one day only a series of related compounds could be tested, each was replicated three to five times. Subsequently, the data obtained on each occasion was subjected to an analysis of variance in order to determine the magnitude of significant difference ($P=0{\cdot}05$) within each series. Although the results of this statistical analysis are omitted from the tables to economize in space, they were kept in mind when writing the general discussion. Tests were invariably repeated—often several times—with broadly concordant results, but only a representative selection of these data is given in Tables 1–5. The object of including numerical data is to give a semi-quantitive picture of toxicity relations rather than to establish accurately the existence of comparatively small toxicity differences, the techniques and variability of the biological material used being such as to preclude the latter. Details of the composition of the toxicants used, and

of the methods employed in their formulation, are described at the appropriate point in the text. Unless otherwise stated, the concentrations referred to in the paper are per cent w/v.

(*a*) *Acaricidal activity of some well-known crop-protection materials*

In the summer of 1946 a survey was made, using the laboratory techniques already referred to, of those crop-protection materials then in use or available for use. These were as follows:

(1) A summer petroleum oil conforming to Ministry of Agriculture specifications (Min. of Agric. 1949).

(2) A powdered barbasco root with a rotenone content of 4·6 %. Calculated amounts were taken to give the rotenone concentration stated.

(3) A colloidal sulphur preparation containing 50 % w/w sulphur, 3% sulphates and the remainder water. The concentrations stated refer to elemental sulphur.

(4) Lime sulphur, sp.gr. 1·3.

The results are summarized in Table 1.

Table 1. *Toxicity of well-known crop-protection materials to summer eggs and adult females*

Material tested	Concentration (%w/w)	Summer eggs (% kill)	Adult females (% kill)
Summer petroleum oil emulsified with 1 % aqueous sodium oleate	1·0*	95·3	62·0
	0·5*	91·0	46·4
	0·25*	76·8	30·3
	0·125*	29·8	16·7
Rotenone (powdered barbasco root) dispersed with 1 % aqueous sodium oleate	0·016	87·3	100·0
	0·008	86·1	100·0
	0·004	67·1	100·0
	0·002	48·1	98·6
	0·001	—	77·9
Summer petroleum oil and rotenone (powdered barbasco root) emulsified with 1 % aqueous sodium oleate	0·5*/0·004	93·0	100·0
	0·35*/0·003	85·2	—
	0·25*/0·002	74·1	100·0
	0·18*/0·0014	56·4	—
	0·125*/0·001	—	97·7
	0·063*/0·0005	52·8	82·8
Lime-sulphur (sp.gr. 1·3), no wetting agent	2·0*	56·6	—
	1·0*	60·5	—
	0·25*	—	98·0
	0·025*	—	15·5
Colloidal sulphur dispersed in 0·025 % w/v Aerosol OT 100	0·5	11·4	—
	0·25	7·3	—
Tetramethylthiuram disulphide†	0·1	79·4	—
	0·05	—	31·3
Di*cyclo*hexylamine-2:4-dinitro-6-*cyclo*-hexylphenate†	0·1	81·3	—
	0·05	74·1	—
	0·025	—	100·0

* Expressed on a v/v basis.

† Dispersed in an aqueous medium containing 4 % acetone, 0·05 % *cyclo*hexylamine dodecyl sulphate and 0·025 % Aerosol OT 100.

Lime sulphur has long been known to afford some degree of control of the red-spider mite, and its failure to effect complete control is believed (Massee, 1930) to be due to its lack of toxicity to the summer egg. This is clearly shown in the results of Table 1 where the percentage kill of adult females and summer eggs with lime sulphur is given. Rotenone is also known to be effective for summer field control, but breaks down unless timing is accurately planned, on account of its lack of toxicity to the egg. Our results suggest that it is not completely non-toxic as some workers have suggested (Garman & Townsend, 1938), and at field concentrations it probably kills more summer eggs than lime sulphur but it nevertheless allows many to escape. Colloidal sulphur is virtually non-toxic. Summer petroleum gives effective control of the eggs, but is only moderately toxic to the adult female. Combined with rotenone, however, summer petroleum gave very high kills of both forms of the mite, a result which is in agreement with observed field performance. However, the use of oil is sometimes limited in certain districts because its phytotoxity prevents its employment at the necessary concentration to give commercial control. As would be expected from the findings of Kagy & McCall (1941), although the di*cyclo*hexylamine salt of 2:4-dinitro-6-*cyclo*hexylphenol was very toxic to the mite, it was not sufficiently toxic to the summer egg to give adequate control at the concentration normally used in the field. Finally attention is drawn to the results given by tetramethylthiuram disulphide, a well-known fungicide. Although this substance was only moderately active, the results suggested that an investigation of the toxicity of related thiuram disulphides would be worth undertaking.

(*b*) *Toxicity of synthetic organic compounds to the summer stages*

The paucity of information on the acaricidal activity of synthetic organic compounds when this investigation started was such as to make it difficult to select a suitable starting-point. The most promising method, however, seemed to be to choose as 'reference compounds' a few substances known to possess appreciable toxicity and then study a number of classes of compounds, the members of each class being related more or less closely to one or other of the reference compounds. The substances examined fall into the following groups:

(1) The 'diphenyl' class. This is a somewhat heterogeneous group of compounds divisible, however, into a number of well-defined series and having azobenzene as their common reference compound.

(2) The alkyl thiocyanate class. Reference compound: *n*-dodecyl thiocyanate.

(3) The organic sulphur class. Reference compound: tetramethylthiuram disulphide.

(4) The organo-phosphorus class. Reference compound: *p*-nitrophenyl diethyl thionphosphate. This work has already been published separately by Eaton & Davies (1950).

(5) The nitrophenol class. Reference compound: di*cyclo*hexylamine salt of 2:4-dinitro-6-*cyclo*hexylphenol. Little work has been done on this class.

All the chemicals tested were pure substances, the melting-point of the specimens used being recorded in the toxicity tables. Some of the compounds were obtained from the usual laboratory sources and were purified where necessary before use. Others were synthesized in this laboratory by methods described in the literature. In the case of the dithiocarbamates and thiuram disulphides their authenticity was established by semi-micro nitrogen analysis. We are indebted to the Shell Petroleum Company for a sample of C_{12}–C_{13} alkyl thiocyanates (*ex* oxo alcohols); the samples of the other thiocyanates tested were identical with those described by Shaw & Steer (1939). The substituted diphenyl sulphones were supplied by courtesy of the Geigy Co. Ltd.; the sample of bis-(*p*-chlorophenyl)methyl carbinol was kindly supplied by the Sherwin-Williams Company and bis-(*p*-chlorophenoxy)methane by the Dow Chemical Company. With the exceptions mentioned below, the substances were formulated in the same way to give dilute test-washes containing the required concentration of toxicant dispersed in 4 % v/v acetone, 0·05 % w/w *cyclo*hexylamine dodecyl sulphate (product MB 320) and 0·025 % w/w dioctyl sodium sulphosuccinate (Aerosol OT 100). To do this the necessary quantity of the chemical under test was weighed into a $3 \times \frac{1}{2}$ in. test-tube and dissolved in 4 ml. of emulsifier-wetter concentrate (1·25 % w/v MB 320 and 0·625 % w/v Aerosol OT 100 in pure acetone). The clear solution was then poured into 96 ml. of distilled water just before dipping of the leaf to give a suspension or emulsion of the toxicant. Because of the low acetone solubility of di*cyclo*pentamethylenethiuram mono-, di-, and hexa-sulphides, it was found necessary to change the solvent, and 1 % v/v pyridine was substituted for the 4 % acetone. The concentration of wetter and emulsifier however remained unchanged. In all tables the concentrations are expressed in terms of the pure substances. Tables 2–4 indicate the results obtained, it will be noted that Table 2 has been subdivided into eight sections. With one or two exceptions, each section deals with compounds having the same number of carbon or other linkage atoms between the two benzene nuclei, thus allowing the effect on toxicity of small changes in molecular configuration to be followed. Of the many conclusions that can be drawn from these tables, the more important are indicated in the Discussion.

Table 2. *The 'diphenyl' class of compounds as summer acaricides. Effect on toxicity of altering the bridging group between the two benzene nuclei and of substitution in the nuclei.*

Substances tested	Concentration (% w/w)	Summer eggs (% kill)	Adult females (% kill)
(1) Diphenyl series:			
	0·1	10·7	14·4
	0·05	—	12·4
	0·025	0·0	—
Diphenyl (m.p. 71·5° C.)			
	0·1	0·0	0·0
	0·025	0·0	0·0
4:4′-Diaminodiphenyl (m.p. 128° C.)			

TABLE 2 (*continued*)

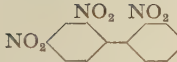
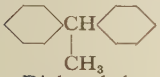
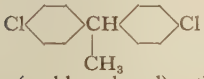
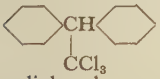
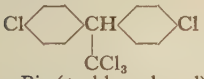
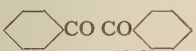
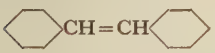
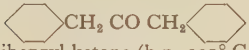
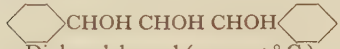
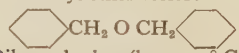
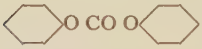
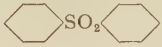
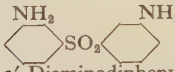
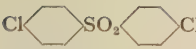
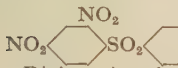
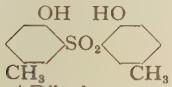
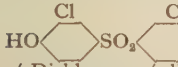
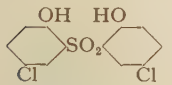
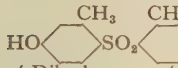
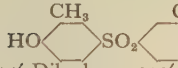
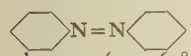
Substances tested	Concentration (% w/w)	Summer eggs (% kill)	Adult females (% kill)
 2:2′:4:4′-Tetranitrodiphenyl (m.p. 65° C.)	0·1	39·8	—
	0·025	14·8	27·4
(2) Diphenylmethane series:			
Diphenyl methane (b.p. 260° C.)	0·1	0·0	47·2
	0·025	0·0	—
Benzophenone (m.p. 45° C.)	0·1	0·6	57·1
Diphenylcarbinol (m.p. 69° C.)	0·1	6·9	38·9
	0·025	2·3	—
 1:1-Diphenylethane (b.p. 272° C.)	0·1	0·0	56·5
	0·025	0·0	—
 Bis-(*p*-chlorophenyl)methyl carbinol (m.p. 68–9° C.)	0·1	97·0	100·0
	0·025	87·9	89·5
 2:2-diphenyl-1:1:1-trichloroethane (m.p. 67° C.)	0·1	37·3	59·0
	0·025	2·8	29·6
 2:2-Bis-(*p*-chlorophenyl)-1:1:1-trichloroethane (D.D.T.) (m.p. 107° C.)	0·1	0·0	50·9
	0·025	0·0	38·3
(3) Dibenzyl series:			
Dibenzyl (m.p. 57° C.)	0·1	0·0	59·1
	0·05	—	33·0
	0·025	0·0	26·2
 Benzil (m.p. 96° C.)	0·1	0·1	65·7
	0·025	0·0	23·4
Benzoin (m.p. 137° C.)	0·1	1·3	41·5
	0·05	—	31·8
	0·025	1·1	15·8
 Stilbene (m.p. 124° C.)	0·1	6·7	54·2
	0·05	—	49·5
	0·025	0·0	27·4

Table 2 (*continued*)

Substances tested	Concentration (% w/w)	Summer eggs (% kill)	Adult females (% kill)
(4) Dibenzoylmethane series:			
Dibenzoylmethane (m.p. 79° C.)	0·1	5·2	12·0
	0·025	2·3	—
 Dibenzyl ketone (b.p. 330° C.)	0·1	8·2	31·8
	0·05	4·7	—
Phenylstyryl ketone (m.p. 61° C.)	0·1	34·4	91·5
	0·05	18·1	65·0
	0·025	0·0	50·2
 α:γ-Diphenylglycerol (m.p. 127° C.)	0·025	0·0	—
(5) Dibenzyl ether series:			
 Dibenzyl ether (b.p. 295° C.)	0·1	3·8	0·0
	0·025	0·0	—
Benzyl benzoate (b.p. 320° C.)	0·1	5·0	84·0
	0·1	—	86·3*
	0·025	0·0	28·6
	0·025	—	60·4*
 Diphenyl carbonate (m.p. 79° C.)	0·1	4·4	71·2
	0·1	—	76·6*
	0·025	0·0	50·5
	0·025	—	49·6*
Bis-(*p*-chlorophenoxy)methane (m.p. 69° C.)	0·1	57·8	87·8
	0·1	—	91·8*
	0·025	28·9	56·2
	0·025	—	53·1*
(6) Diphenylsulphone series:			
 Diphenylsulphone (m.p. 125° C.)	0·1	98·1	69·3
	0·1	—	78·0*
	0·025	83·3	63·1
	0·025	—	66·8*
4-Chlorodiphenylsulphone (m.p. 98° C.)	0·1	96·0	80·9
	0·1	—	81·8*
	0·025	75·9	65·6
	0·025	—	75·6*
4:4′-Dichlorodiphenylsulphone (m.p. 147° C.)	0·1	0·0	—
	0·025	0·0	54·1
	0·025	0·0	52·1*
 3:3′-Diaminodiphenylsulphone (m.p. 169° C.)	0·1	0·0	10·8
	0·025	0·0	—

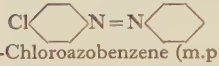
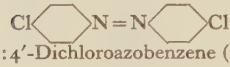
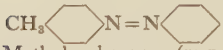
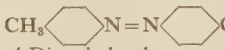
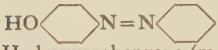
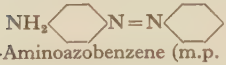
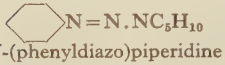
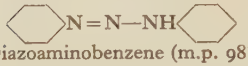
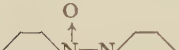
* These mortality results were recorded 48 hr. after treatment instead of the usual 24 hr.

TABLE 2 (*continued*)

Substances tested	Concentration (% w/w)	Summer eggs (% kill)	Adult females (% kill)
 4-Chloro-4′-methyl diphenylsulphone (m.p. 80° C.)	0·1	30·7	50·3
 2:4-Dinitro-4′-methyl diphenylsulphone (m.p. 185° C.)	0·1	0·0	30·8
 2:2′-Dihydroxy-5 : 5′-dimethyl diphenylsulphone (m.p. 197° C.)	0·1	0·0	8·6
 3:3′-Dichloro-4:4′-dihydroxy diphenylsulphone (m.p. 187° C.)	0·1	3·9	45·0
 5:5-Dichloro-2:2′-dihydroxy diphenylsulphone (m.p. 180° C.)	0·1	4·4	50·8
 4:4′-Dihydroxy-2:2′-dimethyl diphenylsulphone (m.p. 129° C.)	0·1	0·0	24·8
 4:4′-Dihydroxy-3:3′-dimethyl diphenylsulphone (m.p. 263° C.)	0·1	0·0	7·5
Diphenylsulphide (b.p. 275° C.)	0·1 0·025	34·0 2·6	50·3 —
Diphenyldisulphide (m.p. 66° C.)	0·1 0·025	0·0 0·0	41·3 22·7
(7) Azobenzene series:			
 Azobenzene (m.p. 60° C.)	0·1 0·1 0·05 0·025 0·025	98·6 — 90·5 73·6 —	75·7 72·6* 67·7 62·0 60·9*

* These mortality results were recorded 48 hr. after treatment instead of the usual 24 hr.

TABLE 2 (*continued*)

Substances tested	Concentration (% w/w)	Summer eggs (% kill)	Adult females (% kill)
 4-Chloroazobenzene (m.p. 89° C.)	0·1	96·8	90·6
	0·1	—	93·4*
	0·025	88·8	37·8
	0·025	—	75·5*
 4:4′-Dichloroazobenzene (m.p. 182° C.)	0·025	8·0	35·7
 4-Methylazobenzene (m.p. 71·5° C.)	0·1	77·3	59·2
	0·1	—	62·4*
	0·025	41·8	12·2
	0·025	—	29·2*
 4:4′-Dimethylazobenzene (m.p. 141·5° C.)	0·1	14·3	0·0
	0·025	0·0	—
3:3′-Dimethylazobenzene (m.p. 53° C.)	0·1	7·5	27·3
	0·025	0·0	0·0
 4-Hydroxyazobenzene (m.p. 149° C.)	0·1	0·0	0·0
	0·025	0·0	—
 4-Aminoazobenzene (m.p. 120° C.)	0·1	68·8	9·5
	0·025	52·6	7·7
 *N*-(phenyldiazo)piperidine (m.p. 44° C.)	0·1	42·6	59·8
	0·025	22·8	31·8
Benzene-azo-*o*-cresol (m.p. 128° C.)	0·5	0·0	4·0
 Diazoaminobenzene (m.p. 98° C.)	0·1	47·8	19·2
	0·025	44·8	2·7
	0·0125	17·1	—
Hydrazobenzene (m.p. 127·5° C.)	0·1	96·3	—
	0·1	—	67·6*
	0·025	93·3	43·8
	0·025	—	51·6
	0·0125	76·0	—
 Azoxybenzene (m.p. 37° C.)	0·1	90·5	—
	0·1	—	100·0*
	0·025	83·1	96·6*
	0·0125	77·0	—

* These mortality results were recorded 48 hr. after treatment instead of the usual 24 hr.

TABLE 2 (*continued*)

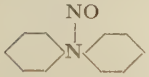
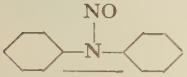
Substances tested	Concentration (% w/w)	Summer eggs (% kill)	Adult females (% kill)
(8) Diphenylamine series:			
Diphenylamine (m.p. 53° C.)	0·1	36·5	32·5
	0·05	31·0	—
	0·025	1·9	—
 *N*-nitroso-diphenylamine (m.p. 67° C.)	0·1	95·3	—
	0·05	83·9	45·6
	0·025	17·5	10·2
 *N*-nitrosocarbazole (m.p. 81·5° C.)	0·1	36·4	20·9
	0·025	4·4	—
Dibenzylamine (b.p. 300° C.)	0·1	7·2	67·8
	0·025	0·7	26·8

TABLE 3. *The alkyl thiocyanates. Effect on toxicity of increasing the number of carbon atoms in the alkyl chain*

Thiocyanate tested	Concentration (% w/w)	Summer eggs (% kill)	Adult females (% kill)
sec.-Hexyl thiocyanate (b.p. *c.* 65° C./4 mm.)	0·1	0·0	0·0
sec.-Heptyl thiocyanate (b.p. *c.* 80° C./4 mm.)	0·1	0·0	4·9
sec.-Octyl thiocyanate (b.p. *c.* 95° C./4 mm.)	0·1	0·0	21·1
sec.-Nonyl thiocyanate (b.p. *c.* 110° C./4 mm.)	0·1	0·0	36·7
sec.-Decyl thiocyanate (b.p. *c.* 125° C./4 mm.)	0·1	31·0	61·4
	0·025	0·0	56·3
sec.-Undecyl thiocyanate (b.p. *c.* 148° C./4 mm.)	0·1	55·7	98·0
	0·025	6·2	50·8
sec.-Dodecyl thiocyanate (b.p. *c.* 155° C./4 mm.)	0·1	73·9	98·2
	0·025	23·9	50·0
tert.-Butyl thiocyanate (b.p. 127° C.)	0·1	0·0	9·1
β-Butoxy-*β*′-thiocyanodiethyl ether (75 % active material)	0·1	0·0	4·8
n-Dodecyl thiocyanate (b.p. 170–2° C./10 mm.)	0·1	86·9	96·8
	0·1	—	98·5*
	0·025	66·1	0·0
	0·025	—	5·7*
(C_{12}–C_{13}) *n*-alkyl thiocyanates (*c.* 60 % active material)	0·1	86·0	96·6
	0·025	28·1	4·4
Cetyl thiocyanate (b.p. 222–7° C./13 mm.)	0·1	88·5	22·6
	0·025	34·8	8·1

* These mortality results were recorded 48 hr. after treatment instead of the usual 24 hr.

Table 4. *Organic sulphur compounds. Effect of changes in molecular structure on the toxicity of certain types of sulphur compounds*

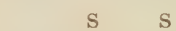
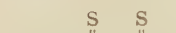
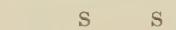
Substances tested	Concentration (% w/w)	Summer eggs (% kill)	Adult females (% kill)
(1) Thiuram disulphide series:			
Dimethylthiuram disulphide (m.p. 105° C.)	0·1	24·4	—
	0·05	—	56·2
 Tetramethylthiuram disulphide (m.p. 155° C.)	0·1	79·4	—
	0·05	99·5	31·3
	0·025	—	40·8
	0·0125	95·2	—
Tetraethylthiuram disulphide (m.p. 75° C.)	0·1	94·3	—
	0·05	92·2	41·1
	0·025	—	63·5
	0·0125	71·0	—
 Tetramethylthiuram monosulphide (m.p. 107–8° C.)	0·1	80·2	—
	0·05	72·3	38·7
	0·025	—	38·7
	0·025	2·8	—
N-diphenyl *N*-dimethylthiuram disulphide (m.p. 199° C.)	0·1	6·5	—
	0·05	—	4·0
N-diphenyl *N*-diethylthiuram disulphide (m.p. 178° C.)	0·1	15·5	—
	0·05	—	16·0
Di*cyclo*pentamethylenethiuram monosulphide (m.p. 121° C.)	0·1	79·7	—
	0·05	21·7	21·8
	0·025	—	—
	0·0125	2·5	—
 Di*cyclo*pentamethylenethiuram disulphide (m.p. 128° C.)	0·1	90·5	—
	0·05	42·2	42·0
	0·025	—	—
	0·0125	9·2	—
Di*cyclo*pentamethylenethiuram hexasulphide (m.p. 134–135° C.)	0·1	72·4	—
	0·05	15·1	77·6
	0·0125	8·8	—

TABLE 4 (*continued*)

(2) Dithiocarbamate series:			
$C_6H_5CH_2NHC(=S)SH.NH_2CH_2C_6H_5$ Benzylamine benzyl dithiocarbamate (m.p. 122° C.)	0·1 0·025	10·0 —	— 24·0
$(C_2H_5)_2NC(=S)SH.NH(C_2H_5)_2$ Diethylamine diethyl dithiocarbamate (m.p. 82° C.)	0·1 0·025	21·8 —	— 0·5
(3) Sulphides and mercaptans:			
$(C_4H_9)_2S$ *n*-Butyl sulphide (b.p. 182° C.)	0·1 0·025	0·0 —	— 7·1
$(C_4H_9)_2S_2$ *n*-Butyl disulphide (b.p. 225° C.)	0·1 0·025	0·0 —	— 0·0
C_4H_9SH *n*-Butyl mercaptan (b.p. 95° C.)	0·1 0·025	1·7 —	— 14·0
C_6H_5SH Thiophenol (b.p. 169° C.)	0·1 0·025	6·8 —	— 6·0

(*c*) *Toxicity of synthetic organic compounds to the winter eggs*

The overwintering eggs attached to shoots of apple seedlings were used in these tests. The testing method used has been described by Eaton & Davies (1950). The melting-point of the pure specimens used are recorded in Table 5. Since no synthetic compounds of appreciable toxicity to the winter eggs were known when the present work began, the tests were made more with the object of selecting substances which might serve as 'reference compounds' in future work than of attempting to relate toxicity to chemical constitution. With the exception of the petroleum oil, all the substances were formulated in the same way to give dilute test-washes containing the required percentage toxicant by weight dispersed in 4 % v/v acetone and 0·05 % w/w *cyclo*hexylamine dodecyl sulphate (product MB 320).

The spindle-type petroleum oil conformed to the Ministry of Agriculture and Fisheries specification for a winter petroleum oil (Min. of Agric. 1949).

The emulsion containing 3 % by volume of petroleum oil was formulated with 0·1 % by weight of MB 320. To do this 6 ml. of Shell JD 2 oil and 0·2 g. of MB 320 were warmed gently until the emulsifier dissolved. A little distilled water was added and the mixture passed through a hand emulsifying mill. The emulsion was then made up to 200 ml. with distilled water. The emulsion containing 1·5 % by volume of petroleum oil was prepared from the above by dilution with distilled water.

Table 5. *Toxicity of some organic compounds to the winter eggs*

Substance	Concentration (%)	Percentage kill (control = 0)
Spindle-type petroleum oil	3·0*	100·0
	1·5*	97·3
n-Dodecyl thiocyanate (b.p. 170–2° C./10 mm.)	0·4	100·0
	0·2	98·5
Azoxybenzene (m.p. 37° C.)	0·2	100·0
	0·05	87·6
Di*cyclo*hexylamine-2:4-dinitro-6-*cyclo*hexylphenate (m.p. 190° C.)	0·2	99·0
	0·05	78·6
Benzaldehyde phenylhydrazone (m.p. 156° C.)	0·2	33·7
	0·05	2·1
4-Aminoazobenzene (m.p. 120° C.)	0·2	28·2
	0·05	7·8
Azobenzene (m.p. 60° C.)	0·2	25·6
	0·05	12·6
Hydrazobenzene (m.p. 127° C.)	0·2	9·3
	0·05	4·0
4-Hydroxyazobenzene (m.p. 149° C.)	0·2	4·1
	0·05	2·0

* Expressed on a v/v basis.

The following substances were examined but showed no appreciable toxicity at 0·2 %: diphenyl methane (b.p. 260° C.); stilbene (m.p. 124° C.); diphenyl sulphone (m.p. 125° C.); dibenzylamine (b.p. 300° C.); benzyl benzoate (b.p. 320° C.); phenyl benzoate (m.p. 70° C.); dibutyl phthalate (b.p. 340° C.); *p*-chlorophenyl benzoate; *N*-nitrosodiphenylamine (m.p. 67° C.); dimethylthiuram disulphide (m.p. 105° C.); tetramethylthiuram disulphide (m.p. 155° C.); di*cyclo*pentamethylenethiuram hexasulphide (m.p. 134–5° C.); dimethyldiphenylthiuram disulphide (m.p. 199° C.); diethyldiphenylthiuram disulphide (m.p. 178° C.); *N*-dimethyl-*S*-dimethyl*iso*thiuram disulphide (b.p. 98° C./10 mm.); 2-mercaptobenzthiazole (m.p. 179° C.); methyl dixanthogen; 1-thiocyano-2:4-dinitrobenzene (m.p. 245° C.); 2:2-diphenyl-1:1:1-trichloroethane (m.p. 67° C.).

Discussion

A group of fifty compounds, characterized by two benzene nuclei in the molecule, was tested against the summer eggs on the one hand and the adult female stage on the other. The figures in Table 2 indicate that there is no well-defined parallelism between the toxicity of the compounds to these two stages. There are, however, individual exceptions to this: notably, bis-(*p*-chlorophenyl)methyl carbinol, 4-chlorodiphenylsulphone, 4-chloroazobenzene and azoxybenzene are highly toxic to both stages, whereas 4-hydroxyazobenzene, 4:4′-diaminodiphenyl, dibenzyl ether and benzeneazo-*o*-cresol have little effect on either stage.

The effect on toxicity of alterations in the bridging group between the two

unsubstituted benzene nuclei is also shown in Table 2, from which it is clear that the nature of this group can have a marked effect on toxicity. For example, replacement of the $—SO_2—$ bridge by the —S— group produces a large and significant fall in toxicity to the summer egg. On the other hand, a variety of bridging groups are associated with appreciable toxicity to the summer stages. Table 6 shows the more toxic linkages.

TABLE 6. *Effect on toxicity of alterations in the bridging group between two unsubstituted benzene nuclei*

	Bridging group between two unsubstituted benzene nuclei	
Toxicity	Summer egg	Adult female
Greater than 90% kill at a concentration 0·1% w/w	—N=N—	O ↑ —N=N—
	$—SO_2—$	
	—NH—NH—	
	NO | —N—	—CH=CH—CO—
	O ↑ —N=N—	

It will be seen that under the conditions of our tests only azoxybenzene was highly toxic to both summer eggs and adult females. However, diphenyl sulphone, azobenzene, hydrazobenzene and *N*-nitrosodiphenylamine were highly efficient summer ovicides while phenylstyryl ketone was highly toxic to the adult female mite.

The effect of substitution in the benzene nuclei has been studied in the azobenzene and the diphenylsulphone series. When azobenzene is substituted in one aromatic nucleus only, the results show that the 4-chloro substituent is as toxic as the unsubstituted azobenzene to the summer egg, followed in order of toxicity by the 4-methyl, 4-amino and finally 4-hydroxy substituent, the last named being quite ineffective. The same order of toxicity was observed in tests carried out against the adult female mite. Substitution in the 4-position in both benzene nuclei also gave interesting results. Thus 4:4′-dichloroazobenzene was much less toxic than 4-chloroazobenzene. This significant reduction in toxicity observed when the 4-chloroazobenzene was further substituted to give the 4:4′-dichloro-body was not an isolated case, a similar effect being noted with the substituted diphenyl sulphones. Whether the effect is still more general and whether it results from an alteration in the effectiveness of the molecule as a cell poison or in the rate at which it penetrates the integument of the mite, remain questions for further study.

It will be noted that bis-(*p*-chlorophenyl)methyl carbinol is a highly potent acaricide and that it bears a structural resemblance to D.D.T., which is known to have rather poor acaricidal properties. Metcalfe (1948), who investigated the reasons for this low toxicity of D.D.T. using *Paratetranychus citri* McG. concluded that the trichlorethane portion of the D.D.T. molecule was associated with low

toxicity and that successive elimination of Cl atoms from this grouping caused a progressive increase in acaricidal activity. However, Metcalfe's theory cannot be generalized to cover any type of substituted trichlorethane derivative, since the compounds

were similar in toxicity to the adult female, while to the summer eggs the former was significantly more toxic, these results being at variance with those expected if Metcalfe's theory were of general application.

The figures in Table 3 indicate the toxicity of a number of alkyl thiocyanates. The observation of Shaw & Steer (1939), that there was a reduction in toxicity to eggs of *Aphis pomi* Deg. and the Vapourer moth, *Orgyia antiqua* (L.), in passing from the primary to the secondary series, was also observed in the present investigation with *Metatetranychus ulmi* summer eggs, but the reduction in toxicity was not nearly so marked, and in the case of the adult female mite no differences could be detected. It is interesting to find that the higher *sec.*-alkyl thiocyanates are appreciably toxic to this pest, thus *sec.*-dedecyl thiocyanate at a concentration of 0·1 % w/w, killed about 74 % of summer eggs and 98 % of adult females, whereas *n*-dodecyl thiocyanate at the same concentration killed 86 % of the summer eggs and 97 % of the adult females.

The most striking effect noticed in this series of *sec.*-alkyl thiocyanates is the increase in toxicity with increasing chain length. It appears that for the adult female this toxicity reaches a maximum about the C_{12} compound but the results for the summer eggs, though not conclusive, suggest that toxicity may not have reached its peak at C_{12}, and further investigation would be desirable to determine whether or not it could be increased by further lengthening the chain. Of the other thiocyanates examined, the high toxicity to both stages of the *n*-dodecyl thiocyanate from vegetable sources and the C_{12}–C_{13} alkyl thiocyanates (*ex* oxo-alcohols) is of interest. At a time of shortage of higher alcohols from natural sources, the high toxicity of the synthetic compound is of considerable importance. Attention is also drawn to the low activity of β-butoxy-β'-thiocyanodiethyl ether, a compound which was known to be ineffective against the winter eggs of *M. ulmi* (Bennett *et al.* 1947), but which Shaw & Steer had noted as being worthy of extended trials as an ovicide against *Aphis pomi* Deg.

The organic sulphur compounds examined for toxicity as summer acaricides included dithiocarbamates, alkyl sulphides and thiuram disulphides. Of the thiuram disulphides only the tetra-alkyl series proved appreciably toxic. Replacement of two of the alkyl groups by two phenyl groups brings about a marked reduction in toxicity; thus *N*-diphenyl-*N*-dimethylthiuram disulphide is virtually non-toxic at a concentration of 0·1 %. This inactivity may be connected with the stability of the dialkyldiphenyl derivatives which are known (unpublished data) to be much more stable than the tetra-alkyl disulphides. While investigating the toxicity of the thiuram

disulphide series, it was obviously of interest to ascertain the effect of shortening or lengthening the sulphur chain between the two thion groups. Consequently, the toxicity of a series of di*cyclo*pentamethylenethiuram-mono-, di- and hexa-sulphides was examined. The results given in Table 4 indicate that toxicity to the summer eggs is at a maximum with the disulphide, but that toxicity to the adult female stage appears to increase with increasing length of the sulphur chain, the maximum adult female kill observed for the series being given by di*cyclo*pentamethylenethiuram hexasulphide.

Of the many substances tested as summer acaricides the ones which seem most deserving of field investigation are shown in Table 7. Some of these compounds may be phytotoxic, some may be too expensive and others, perhaps most, will be rejected for a variety of reasons. It is already known from preliminary field trials using Cox's Orange Pippin apple trees that all formulations yet tried of azobenzene

TABLE 7. *The most effective summer acaricides as indicated by laboratory toxicity tests*

Substances	Concentration (% w/w)	Summer eggs (% kill)	Adult females (% kill)
Bis-(*p*-chlorophenyl)methyl carbinol (m.p. 68–9° C.)	0·1	97·0	100·0
	0·025	87·9	89·5
4-Chloroazobenzene (m.p. 89° C.)	0·1	96·8	93·4*
	0·025	88·8	75·5*
Azoxybenzene (m.p. 37° C.)	0·1	90·5	100·0*
	0·025	83·1	96·6
n-Dodecyl thiocyanate (b.p. 170–2° C./10 mm.)	0·1	86·9	96·8
	0·025	66·1	0·0
n(C_{12}–C_{13}) alkyl thiocyanates (approx. 60% active material)	0·1	86·0	96·6
	0·025	28·1	4·4
4-Chlorodiphenylsulphone (m.p. 98° C.)	0·1	96·0	80·9
	0·025	73·9	65·6
Azobenzene (m.p. 60° C.)	0·1	98·6	75·7
	0·05	90·5	67·7
	0·025	73·6	62·0
sec.-Dodecyl thiocyanate (b.p. *c.* 155° C./4 mm.)	0·1	73·9	98·2
	0·025	23·9	50·0
Diphenylsulphone (m.p. 127° C.)	0·1	98·1	69·3
	0·025	83·3	63·1
Hydrazobenzene (m.p. 127·5° C.)	0·1	96·3	67·6
	0·025	93·3	43·8
	0·0125	76·0	—
N-nitrosodiphenylamine (m.p. 69° C.)	0·1	95·3	—
	0·05	83·9	45·6
	0·025	17·5	10·2
Bis-(*p*-chlorophenoxy)methane (m.p. 69–70° C.)	0·1	57·8	91·8*
	0·025	28·9	53·1*
Tetraethylthiuram disulphide (m.p. 75° C.)	0·1	94·3	41·1
	0·025	71·0	—

* These mortality results were recorded 48 hr. after dipping instead of the usual 24 hr.

or azoxybenzene at 0·1 % have caused appreciable leaf- and fruit-fall. It is, however, evident that bis-(*p*-chlorophenyl)methyl carbinol, 4-chloroazobenzene, C_{12}–C_{13} alkyl thiocyanates, 4-chlorodiphenylsulphone and diphenylsulphone are worthy of trial in the field.

As already mentioned, the investigation on the toxicity of synthetic organic compounds to the winter egg has not advanced as far as the summer acaricide work. The results in Table 5 represent the progress made in a search for suitable reference compounds. Three compounds (apart from petroleum oil) have appreciable toxicity to the winter eggs. These are *n*-dodecyl thiocyanate, azoxybenzene and di*cyclo*hexylamine-2:4-dinitro-6-*cyclo*hexylphenate. Various workers, including Bennett *et al.* (1947) have stated that petroleum even at 5 % does not completely inhibit the hatch of winter eggs: if this be the case, it is obvious that these three compounds may be worthy of trial as fortifying agents with petroleum. Since these compounds are toxic to both summer and winter stages of the fruit-tree red-spider there may be reasons to investigate their use at the time of the hatch of winter eggs as well as during the summer and the dormant period.

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THE INSECTICIDAL ACTIVITY OF SUBSTANCES RELATED TO THE PYRETHRINS

I. THE TOXICITIES OF TWO SYNTHETIC PYRETHRIN-LIKE ESTERS* RELATIVE TO THAT OF THE NATURAL PYRETHRINS AND THE SIGNIFICANCE OF THE RESULTS IN THE BIOASSAY OF CLOSELY RELATED COMPOUNDS

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(With 13 Text-figures)

The toxicity as contact insecticides of the esters of (±)-3-methyl-2-allyl-*cyclo*pent-2-en-4-ol-1-one with the natural (+)-*trans* and with synthetic (±)-*cis-trans*-chrysanthemum monocarboxylic acids has been compared with that of the natural pyrethrins. The comparison was carried out on five species of test-insects.

It was found that the figure for both the absolute and the relative toxicities of these compounds varied with the species used as test-subject.

The factors involved in this variation are discussed. The extremes of variation of relative toxicity for the compounds with the natural acid were from about one-eighth as toxic as the pyrethrum standard when the aphid *Macrosiphum solanifolii* was used as test subject to nearly four times as toxic as the standard with the larvae of the moth *Plutella maculipennis*. The fully synthetic material varied from about one-sixteenth as toxic as the standard to *Macrosiphum solanifolii* to nearly twice as toxic to the larvae of *Plutella maculipennis*. The compound with the natural acid was approximately twice as toxic as the fully synthetic material to three of the insect species, but the fully synthetic material was considerably more than half as toxic as the partially synthetic to the fourth test-species. It is pointed out that while it is widely recognized that large differences in relative toxicity may occur when the effect of chemicals of widely different structure and mechanism of action are compared on a number of different test-species, the fact that these differences may also occur with related chemicals with, presumably, a similar mechanism of action, has not been clearly stated.

Even when the differences in relative toxicity are taken into account, the two synthetic pyrethrin-like esters still show high insecticidal activity.

It seems reasonable to suppose from the results that economic commercial synthesis of pyrethrin-like insecticides is not impossible.

Introduction

Following the fundamental chemical investigations of Staudinger and Ruzicka, published in 1924, work has been in progress, chiefly in the U.S.A. and in this country, on the structure and possibilities of synthesis of the insecticidal principles of pyrethrum flowers. Detailed reviews of these researches have been given by Harper (1948, 1949).

* These were the esters of 3-methyl-2-allyl-*cyclo*pent-2-en-4-ol-1-one with (+)-*trans* (natural) and (±)-*cis-trans* (synthetic) chrysanthemum monocarboxylic acids respectively.

In the course of this work, LaForge and his collaborators in the U.S.A. recently developed the pyruvaldehyde route to keto alcohols related to cinerolone (Schechter, Green & LaForge, 1949) which, in conjunction with the work of Campbell & Harper (1945), on the synthesis of chrysanthemum monocarboxylic acid, has made it possible to synthesize chemically pure pyrethrin-like materials of high biological activity.

The only detailed quantitative results published on the insecticidal activity of synthetic pyrethrin-like materials are of those of Gersdorff (1947, 1949*a*, *b*). These investigations were limited to one insect species, the adult housefly, *Musca domestica* L., and one medium, namely kerosene. Some further results have been given recently by Stoddard & Dove (1949), using a number of insect species, but in this paper, also, the only quantitative comparisons are those on houseflies in kerosene.

Ever since the early work of Tattersfield and his associates on pyrethrum flowers and the assay of their active principles, the Insecticides Department at Rothamsted has worked on one or more of the special aspects of the pyrethrins as insecticides, and the development of these synthetic compounds was of especial interest to us. First, any synthetic material with properties like those of the pyrethrins, particularly their lack of mammalian toxicity, which could be produced at a competitive price, might have considerable practical importance and prove a useful addition to the insecticides available. Especially is this so in view of the recent trend to produce insecticides which are more and more dangerous to man. Secondly, they provided useful materials for an investigation of their toxicity as part of a study in progress on the relationship of chemical structure to insecticidal activity.

Through the courtesy of Dr H. Kaan of the Chemical-Biological Co-ordination Committee of the National Research Council of the U.S.A. and the kindness of Dr LaForge, we were able to obtain two of these pyrethrin-like substances. One ester, partially synthetic, was the synthetic keto-alcohol (±)-3-methyl-2-allyl-*cyclo*-pent-2-en-4-ol-1-one esterified with the naturally occurring (+)-*trans*-chrysanthemum monocarboxylic acid, and the other was the wholly synthetic ester of the same keto alcohol with the synthetic mixture of (±)-*cis*-*trans*-chrysanthemum monocarboxylic acids (Campbell & Harper, 1945). The partially synthetic material was the most toxic to houseflies of twelve chemically pure compounds closely related to cinerin I which were tested by Gersdorff (1949*a*), and the fully synthetic material was also highly active.

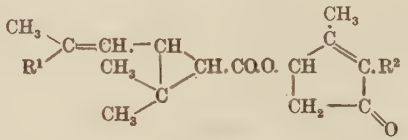
The work described in this paper consists of a comparison of the activities of these two esters as contact insecticides with that of the natural pyrethrins, to five species of insects, four of them plant feeding and one a stored products pest. The object was to determine how effective the materials were when applied as contact poisons in an aqueous medium against a number of species of insects, particularly those feeding on plants, and to compare the results with those of Gersdorff, who used a kerosene medium.

It was found that both the absolute toxicity and the toxicity relative to the pyrethrins varied considerably with the test-subject.

This difference in relative toxicity with species was high considering that the esters were so closely related, and is discussed more fully later. The results therefore seem to have some importance when considering the significance of any comparative bio-assay of chemical compounds of related structure. This work is not regarded as a completed study and these preliminary results are submitted on this account, and because the materials may be of early practical importance.

MATERIALS

(*a*) *Synthetic esters.* The samples, dispatched from the U.S.A. on 3 June 1949 and received on 24 June 1949, were stored in a refrigerator at approximately 2° C. in the dark, except when samples were taken. Their formulae are shown in Fig. 1, together



Compound		R^1	R^2	Configuration of acid	Configuration of keto alcohol
Natural pyrethrins	Pyrethrin I	CH_3	$.CH_2CH{=}CH.CH{=}CH_2$	(+)-*trans*	(+)
	Pyrethrin II	CH_3OOC	$.CH_2CH{=}CH.CH{=}CH_2$	(+)-*trans*	(+)
	Cinerin I	CH_3	$.CH_2CH{=}CH.CH_3$	(+)-*trans*	(+)
	Cinerin II	CH_3OOC	$.CH_2CH{=}CH.CH_3$	(+)-*trans*	(+)
Partially synthetic ester		CH_3	$.CH_2CH{=}CH_2$	(+)-*trans*	(±)
Completely synthetic ester		CH_3	$.CH_2CH{=}CH_2$	(±)-*cis-trans*	(±)

Fig. 1. Accepted structural formulae of the compounds tested.‡

with those of the pyrethrins for the purpose of comparison. The substances were analysed before and after the period of the tests as a possible check on deterioration.*

The following data on the compounds were obtained.

(±)-allylrethronyl (+)-*trans*-chrysanthemate:†

A pale brown liquid n_D^{25} 1·5047

(*a*) 29 June 1949	C = 75·91 : 75·34 %
	H = 9·24 : 9·10 %
(*b*) 10 November 1949	C = 75·29 %
	H = 9·47 %
$C_{19}H_{26}O_3$ requires	C = 75·46 %
	H = 8·67 %

* Analyses carried out by Drs Weiler and Strauss, Micro-analytical Laboratory, Oxford.

† The nomenclature used throughout this section follows Harper, *Chemistry and industry*, p. 636, 10 September 1949.

‡ No consideration of the stereochemistry of the side chain is made here. For a discussion of this subject see Harper (1948, 1949) and Crombie & Harper (1950).

(±)-allylrethronyl (±)-*cis-trans*-chrysanthemate:

A very pale yellow liquid n_D^{21} 1·5070

(*a*) 15 September 1949 C = 74·34 : 74·64 %
H = 9·03 : 8·47 %

(*b*) 10 November 1949 C = 75·49 %
H = 9·23 %

$C_{19}H_{26}O_3$ requires C = 75·46 %
H = 8·67 %

(*b*) *Pyrethrins*. The solutions of natural pyrethrins, isolated by the nitromethane technique, were prepared from a petroleum ether (b.p. 40–60° C.) solution containing 3 % total pyrethrins. The stock solution gave the following analyses, using a modified Wilcoxon-Holaday mercury-reduction method for pyrethrin I and the Seil method for pyrethrin II.

Pyrethrin I	1·86 %		
Pyrethrin II	1·15 %		
Total pyrethrins	3·01 %	Total solids	3·33 %

Technique of testing

The substances were applied as contact insecticides in the Potter tower (Potter, 1941). An aqueous medium containing 0·1 % sulphonated lorol and 10 % acetone was used throughout.

The insects were counted into 2 × 1 in. glass tubes on the morning of the day of spraying and kept at room temperature. For spraying, they were placed on circles of 'Tricoline' cloth in 9 cm. Petri dishes and were subsequently stored in the same dishes. The temperature of storage varied from 17 to 20° C. throughout the tests but was approximately constant for each test. The humidity was variable.

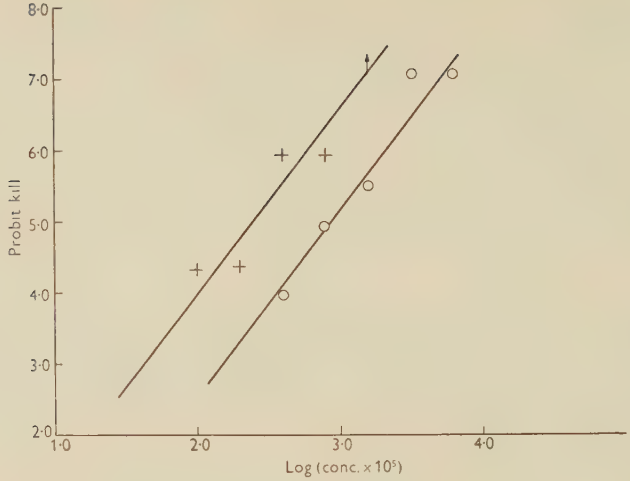
The insects were inspected on warmed plates maintained at a constant temperature to obtain uniform comparisons of activity.

The results were analysed by the probit technique as modified by Finney (1947).

Test-insects

Phaedon cochleariae Fab., mustard beetle. Adult beetles, chosen without selection of male or female, from a culture in which egg laying had commenced. The cultures were reared on turnips in a glasshouse. After treatment they were kept in the spraying dishes for 4 days in the first series of tests (Figs. 5, 6) or for 3 days in the second series (Table 7) and then inspected.

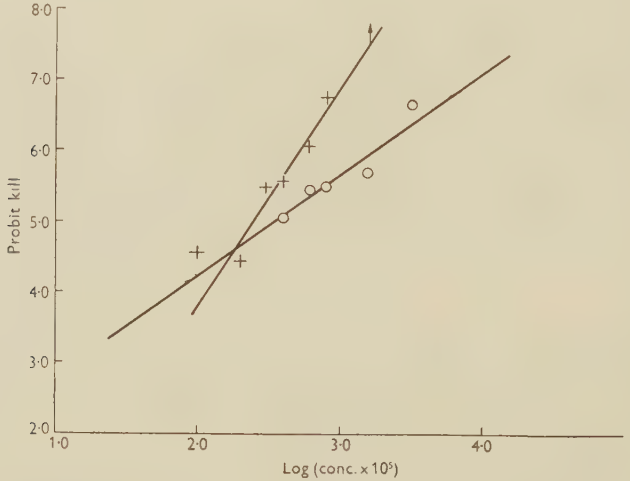
Oryzaephilus surinamensis L., saw toothed grain beetle. Adult beetles were used from cultures reared on rolled oats in an incubator at 29° C. In the first series of tests (Figs. 8, 9) the insects were taken from a 7-week-old culture, and in the second series (Table 8) from a 9-week-old culture. They were kept in the spraying dishes for 2 days after treatment and then inspected.



Equations of probit lines	Log (LD 50 × 10^5)	LD 50 % w/v
A. $Y = 2{\cdot}60x - 2{\cdot}68$	$2{\cdot}95 \pm 0{\cdot}05$	0·00899
B. $Y = 2{\cdot}60x - 1{\cdot}24$	$2{\cdot}40 \pm 0{\cdot}05$	0·00251

Treatment difference is significant.

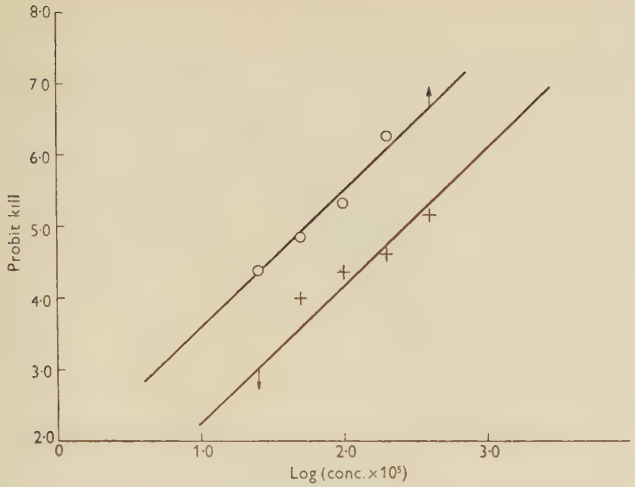
Fig. 2. *Plutella maculipennis* Curtis [8. vii. 49]. ○, extract of natural pyrethrins (A); +, (±)-allylrethronyl (+)-*trans*-chrysanthemate (B); ↑, point at which 100% kill occurred. Slope (b) = 2·60 ± 0·24.



Equations of probit lines	Log (LD 50 × 10^5)	LD 50 % w/v
A. $Y = 1{\cdot}39x + 1{\cdot}47$	$2{\cdot}54 \pm 0{\cdot}20$	0·00346
C. $Y = 2{\cdot}86x - 1{\cdot}81$	$2{\cdot}38 \pm 0{\cdot}05$	0·00241

Treatment difference is not significant at LD 50.

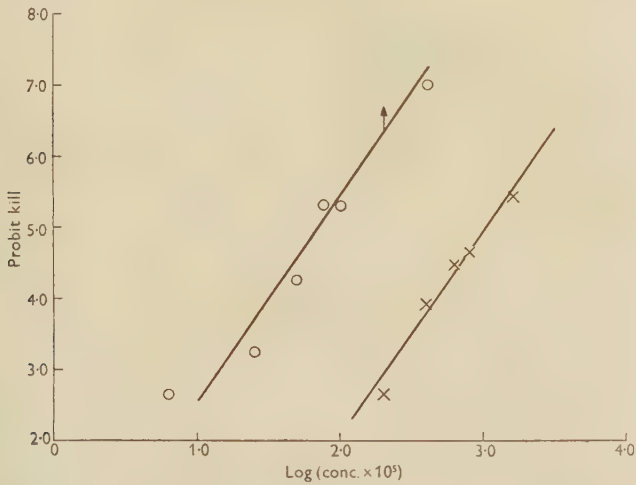
Fig. 3. *Plutella maculipennis* Curtis [27. ix. 49]. ○, extract of natural pyrethrins (A); +, (±)-allylrethronyl (±)-*cis*-*trans*-chrysanthemate (C); ↑, point at which 100% kill occurred. Slopes (b) A, 1·39 ± 0·44; C, 2·86 ± 0·36.



Equations of probit lines		Log (LD 50 × 10^6)	LD 50 % w/v
A.	$Y = 1{\cdot}91x + 1{\cdot}69$	$1{\cdot}73 \pm 0{\cdot}06$	0·000541
B.	$Y = 1{\cdot}91x + 0{\cdot}35$	$2{\cdot}43 \pm 0{\cdot}07$	0·002720

Treatment difference is significant.

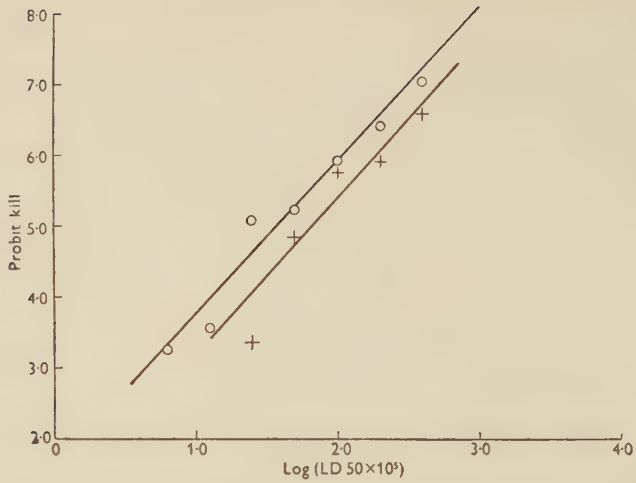
Fig. 4. [*Macrosiphum solanifolii* Ashm. [29. vi. 49]. ○, extract of natural pyrethrins (A); +, (±)-allylrethronyl (+)-*trans*-chrysanthemate (B); ↑, point at which 100% kill occurred; ↓, point at which no kill occurred. Slope (b) = 1·92 ± 0·19.



Equations of probit lines		Log (LD 50 × 10^6)	LD 50 % w/v
A.	$Y = 3{\cdot}02x - 0{\cdot}58$	$1{\cdot}85 \pm 0{\cdot}04$	0·000704
C.	$Y = 3{\cdot}02x - 4{\cdot}05$	$3{\cdot}00 \pm 0{\cdot}04$	0·0092

Treatment difference is significant.

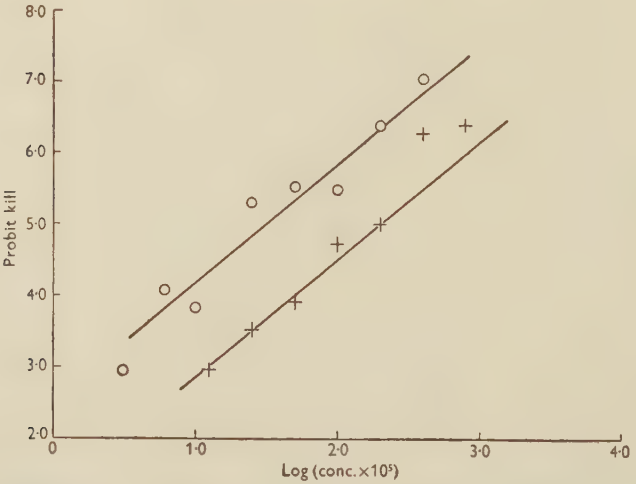
Fig. 5. *Aphis fabae* Scop. [8. ix. 49]. ○, extract of natural pyrethrins (A); ×, (±)-allylrethronyl (±)-*cis-trans*-chrysanthemate (C); ↑, point at which 100% kill occurred. Slope (b) = 3·02 ± 0·32.



Equations of probit lines	Log (LD 50 × 10^5)	LD 50 % w/v
A. $Y = 2{\cdot}18x + 1{\cdot}58$	$1{\cdot}57 \pm 0{\cdot}06$	0·000371
B. $Y = 2{\cdot}18x + 1{\cdot}03$	$1{\cdot}82 \pm 0{\cdot}07$	0·000662

Treatment difference is significant.

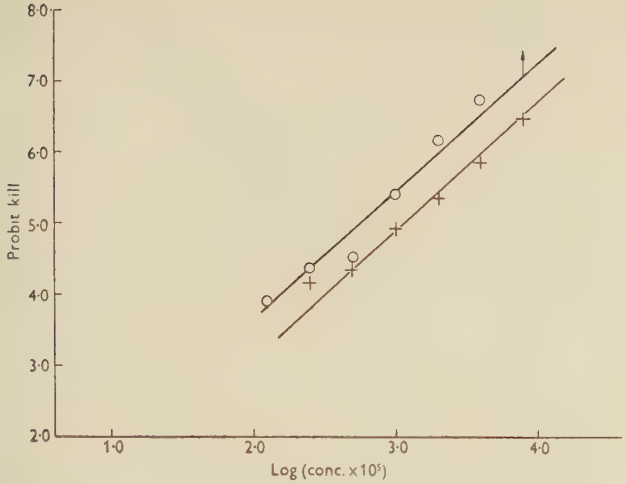
Fig. 6. *Phaedon cochleariae* Fab. [30. vi. 49]. ○, extract of natural pyrethrins (A); +, (±)-allylrethronyl (+)-*trans*-chrysanthemate (B). Slope (b) = 2·18 ± 0·15.



Equations of probit lines	Log (LD 50 × 10^6)	LD 50 % w/v
A. $Y = 1{\cdot}65x + 2{\cdot}55$	$1{\cdot}49 \pm 0{\cdot}08$	0·000305
C. $Y = 1{\cdot}65x + 1{\cdot}20$	$2{\cdot}30 \pm 0{\cdot}08$	0·002009

Treatment difference is significant.

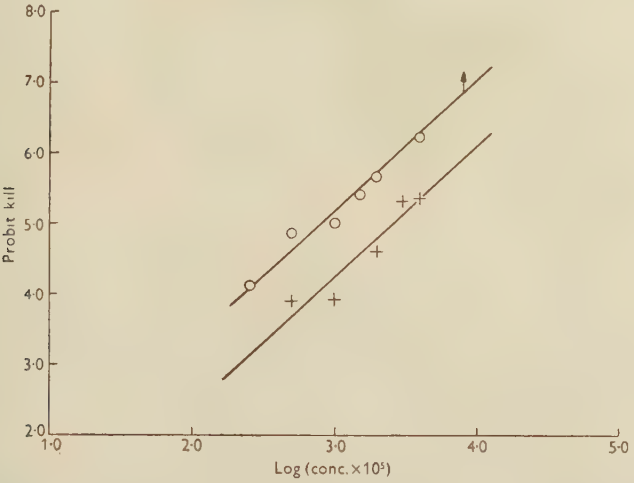
Fig. 7. *Phaedon cochleariae* Fab. [25. viii. 49]. ○, extract of natural pyrethrins (A); +, (±)-allylrethronyl (±)-*cis*-*trans*-chrysanthemate (C). Slope (b) = 1·65 ± 0·11.



Equations of probit lines	Log (LD 50 × 10^8)	LD 50 % w/v
A. $Y = 1{\cdot}82x + 0{\cdot}01$	$2{\cdot}74 \pm 0{\cdot}05$	0·00552
B. $Y = 1{\cdot}82x - 0{\cdot}05$	$3{\cdot}05 \pm 0{\cdot}05$	0·0112

Treatment difference is significant.

Fig. 8. *Oryzaephilus surinamensis* L. [4. x. 49]. ○, extract of natural pyrethrins (A); +, (±)-allylrethronyl (+)-*trans*-chrysanthemate (B); ↑, point at which 100% kill occurred. Slope (b) = 1·82 ± 0·14.



Equations of probit lines	Log (LD 50 × 10^8)	LD 50 % w/v
A. $Y = 1{\cdot}85x - 0{\cdot}36$	$2{\cdot}90 \pm 0{\cdot}04$	0·00789
C. $Y = 1{\cdot}85x - 1{\cdot}32$	$3{\cdot}42 \pm 0{\cdot}05$	0·02607

Treatment difference is significant.

Fig. 9. *Oryzaephilus surinamensis* L. [1. ix. 49]. ○, extract of natural pyrethrins (A); +, (±)-allylrethronyl (±)-*cis-trans*-chrysanthemate (C); ↑, point at which 100% kill occurred. Slope (b) = 1·85 ± 0·15.

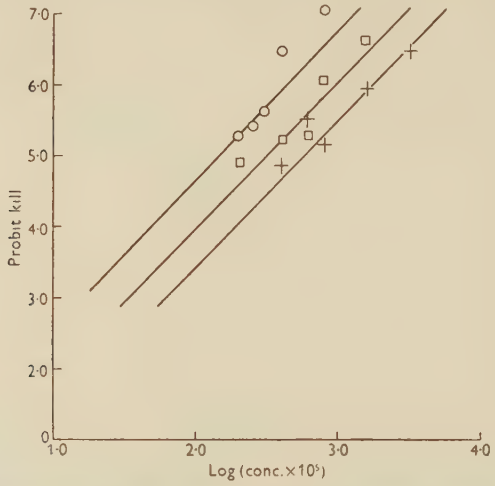


Fig. 10. *Plutella maculipennis* Curtis [10. xi. 49]. +, natural pyrethrins; ○, (±)-allyl-rethronyl (+)-*trans*-chrysanthemate; □, (±)-allylrethronyl (±)-*cis-trans*-chrysanthemate.

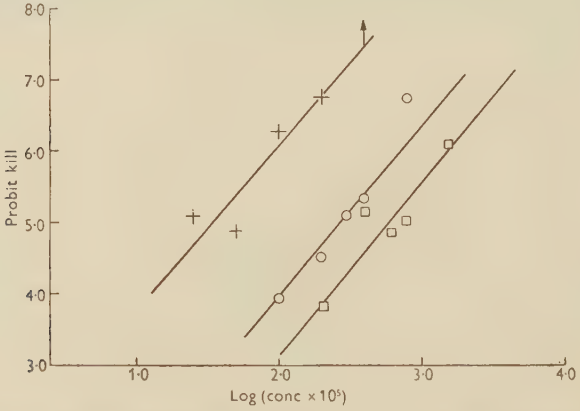


Fig. 11. *Macrosiphum solanifolii* Ashm. [21. xi. 49]. +, natural pyrethrins; ○, (±)-allyl-rethronyl (+)-*trans*-chrysanthemate; □; (±)-allylrethronyl (±)-*cis-trans*-chrysanthemate; ↑, point at which 100% kill occurred.

Plutella maculipennis Curt., diamond back moth. Final instar larvae were used and individuals which had finished feeding were selected as far as possible. The insects were reared on cabbage in a glasshouse. In the first series of tests (Figs. 2, 3) the insects were kept for 3 and 6 days respectively after treatment and then inspected, and in the second series (Table 5) for 5–6 days. Food was added during the storage after spraying in each case.

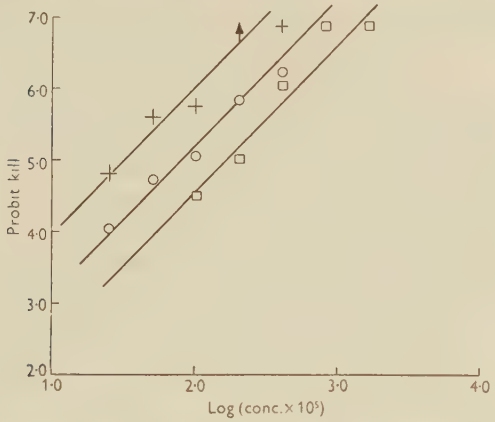


Fig. 12. *Phaedon cochleariae* Fab. [6. xii. 49]. +, natural pyrethrins; ○, (±)-allylrethronyl (+)-*trans*-chrysanthemate; □, (±)-allylrethronyl (±)-*cis*-*trans*-chrysanthemate; ↑, point at which 100% kill occurred.

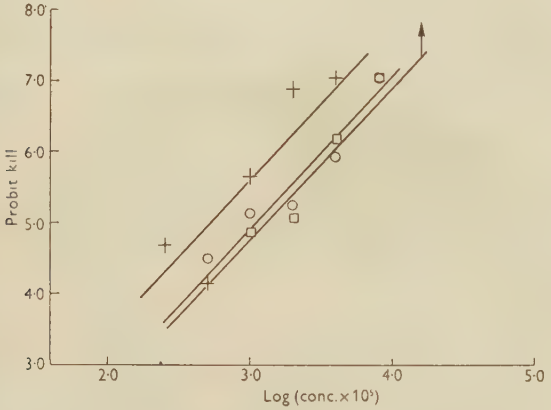


Fig. 13. *Oryzaephilus surinamensis* L. [20. xii. 49]. +, natural pyrethrins; ○, (±)-allylrethronyl (+)-*trans*-chrysanthemate; □, (±)-allylrethronyl (±)-*cis*-*trans*-chrysanthemate; ↑ point at which 100% kill occurred.

Macrosiphum solanifolii Ashm. (=*euphorbiae* Thomas), potato aphid. Adult apterous viviparous parthogenetic females reared in a glasshouse were used. The host plant for the first series of tests (Fig. 4) was aquilegia and for the second series (Table 6) cineraria. After spraying the insects were kept in the spraying dishes for 24 hr. without food and then inspected.

Aphis fabae Scop., bean aphid. Adult apterous viviparous parthogenetic females reared on nasturtiums in a glasshouse were used (Fig. 5.) The test subjects were

kept in the spraying dishes for 24 hr. after treatment without added food, and were then inspected.

Experimental results

Two series of experiments were carried out. In the first the partially synthetic and the wholly synthetic esters were evaluated separately on different days against the test-insects used, with the natural pyrethrins as a standard. The full toxicity data for these experiments is not reproduced here, but the equations to the regression lines and the regression lines themselves are shown in Figs. 2–9, and the relative toxicities derived from the data are set out in Table 3. In the second series of experiments the two synthetics and the standard were all applied to the particular test-subject on the same day and all the conditions of the test were therefore more nearly the same for both materials with any given test-insect. The comparative data obtained in this way are thought to be more reliable than in the earlier series but in fact the results agree well. The full toxicity data for the second series of experiments is set out in Tables 5–8, and the equations to the regression lines derived from this data together with their slopes and L.D. 50's are set out in Table 1 and Figs. 10–13. The relative potencies of the three materials to each species of test-subject are set out in Table 2.

All the probit lines in any one experiment were parallel, with the exception of *Plutella maculipennis*, 27. ix. 49.

The residual χ^2 of heterogeneity was significant in three cases, namely *Oryzaephilus surinamensis*, 20. xii. 49, and *Phaedon cochleariae*, 30. vi. 49 and 25. viii. 49.

Table 1. *Comparison of toxicity of natural pyrethrins* (A), (±)-*allylrethronyl* (+)-trans-*chrysanthemate* (B) *and* (±)-*allylrethronyl* (±)-cis-trans-*chrysanthemate* (C)

(N.B. The following results were obtained by spraying all three compounds against each insect in the same experiment.)

	Equation of probit line	Slope of probit line	Log (LD 50 × 10^5)	LD 50 %w/v
	[10. xi. 49] *Plutella maculipennis* Curtis, diamond back moth			
A.	$Y=2{\cdot}08x-0{\cdot}74$	$2{\cdot}08\pm0{\cdot}05$	$2{\cdot}76\pm0{\cdot}06$	0·005754
B.	$Y=2{\cdot}08x+0{\cdot}50$	$2{\cdot}08\pm0{\cdot}05$	$2{\cdot}16\pm0{\cdot}10$	0·001415
C.	$Y=2{\cdot}08x-0{\cdot}19$	$2{\cdot}08\pm0{\cdot}05$	$2{\cdot}50\pm0{\cdot}06$	0·003162
	[21. xi. 49] *Macrosiphum solanifolii* Ashm., potato aphid			
A.	$Y=2{\cdot}36x+1{\cdot}38$	$2{\cdot}36\pm0{\cdot}27$	$1{\cdot}53\pm0{\cdot}07$	0·00034
B.	$Y=2{\cdot}36x-0{\cdot}75$	$2{\cdot}36\pm0{\cdot}27$	$2{\cdot}44\pm0{\cdot}05$	0·00275
C.	$Y=2{\cdot}36x-1{\cdot}54$	$2{\cdot}36\pm0{\cdot}27$	$2{\cdot}77\pm0{\cdot}06$	0·00589
	[6. xii. 49] *Phaedon cochleariae* Fab., mustard beetle			
A.	$Y=2{\cdot}03x+1{\cdot}93$	$2{\cdot}03\pm0{\cdot}04$	$1{\cdot}51\pm0{\cdot}08$	0·000324
B.	$Y=2{\cdot}03x+1{\cdot}13$	$2{\cdot}03\pm0{\cdot}04$	$1{\cdot}91\pm0{\cdot}08$	0·000813
C.	$Y=2{\cdot}03x+0{\cdot}52$	$2{\cdot}03\pm0{\cdot}04$	$2{\cdot}21\pm0{\cdot}07$	0·001622
	[20. xii. 49] *Oryzaephilus surinamensis* L., saw-toothed grain beetle			
A.	$Y=2{\cdot}15x-0{\cdot}86$	$2{\cdot}15\pm0{\cdot}15$	$2{\cdot}73\pm0{\cdot}08$	0·00537
B.	$Y=2{\cdot}15x-1{\cdot}55$	$2{\cdot}15\pm0{\cdot}15$	$3{\cdot}05\pm0{\cdot}08$	0·01122
C.	$Y=2{\cdot}15x-1{\cdot}70$	$2{\cdot}15\pm0{\cdot}15$	$3{\cdot}12\pm0{\cdot}11$	0·01318

TABLE 2. *Summary of the relative potencies of natural pyrethrins,* (±)-*allylrethronyl* (+)-trans-*chrysanthemate and* (±)-*allylrethronyl* (±)-cis-trans-*chrysanthemate from the experiments referred to in Table* 1

	Plutella maculipennis [10. xi. 49]	*Macrosiphum solanifolii* [21. xi. 49]	*Phaedon cochleariae* [6. xii. 49]	*Oryzaephilus surinamensis* [20. xii. 49]
Nat. pyr.	1000	1000	1000	1000
(+)-*trans*	3980	123	399	479
(±)-*cis-trans*	1820	58	200	406

TABLE 3. *Summary of the relative potencies of natural pyrethrins,* (±)-*allylrethronyl* (+)-trans-*chrysanthemate and* (+)-*allylrethronyl* (−)-cis-trans-*chrysanthemate from earlier, preliminary, experiments where all three compounds were not sprayed in the same test.*

	Plutella maculipennis	*Macrosiphum solanifolii*	*Aphis fabae*	*Phaedon cochleariae*	*Oryzaephilus surinamensis*
Nat. pyr.	1000	1000	1000	1000	1000
(+)-*trans*	3582 [8. vii. 49]	199 [29. vi. 49]	—	560 [30. vi. 49]	493 [4. x. 49]
(±)-*cis-trans*	1436 [27. ix. 49]	—	71 [8. ix. 49]	152 [25. viii. 49]	303 [1. ix. 49]

TABLE 4. *Maximum variations in absolute toxicity*

Substance	Minimum LD 50 % w/v	Maximum LD 50 % w/v	Ratio, maximum LD 50 / minimum LD 50
Natural pyrethrins	*Phaedon cochleariae* 0·000371	*Plutella maculipennis* 0·00899	*c.* 24
Partially synthetic ester	*P. cochleariae* 0·000662	*Oryzaephilus surinamensis* 0·0112	*c.* 17
Completely synthetic ester	*P. cochleariae* 0·00201	*O. surinamensis* 0·0261	*c.* 13

TABLE 5. *Comparison of toxicity of natural pyrethrins* (A), (±)-*allylrethronyl* (+)-trans-*chrysanthemate* (B) *and* (±)-*allylrethronyl* (±)-cis-trans-*chrysanthemate* (C) *against* Plutella maculipennis *Curtis, diamond back moth,* 10. xi. 49

	Dosage (% w/v)	No. of insects*	Mortality (%)	Corrected (% mortality)
A. 1	0·032	59	93	93
2	0·016	57	84	83
3	0·008	61	59	56
4	0·006	60	72	70β
5	0·004	56	48	45
B. 8	0·008	62	98	98
9	0·004	57	93	93
10	0·003	60	75	73
11	0·0025	59	68	66
12	0·002	56	63	61

TABLE 5 (*continued*)

C. 13	0·016	57	95	95
14	0·008	57	86	85
15	0·006	59	63	61
16	0·004	56	61	59
17	0·002	53	49	46
6+18	Sprayed control	153	6	—
7+19	Unsprayed	97		

Slope (b) = 2·08 ± 0·05.

The treatment difference is significant, see Fig. 10.

Spraying conditions: deposit 7·01 mg./cm.^{2}; temperature 23·5° C.; humidity 47·5 % R.H.

Storage of insects after spraying: in the dark at a temperature of 17° C. (approximately) and a humidity of 55 % R.H. (approximately).

* These figures represent four replicates of about fifteen insects at each concentration.

With the exception of the anomalous point indicated (β) the data could be fitted by three parallel lines.

Equation of probit lines	Log (LD 50 × 10^5)
A. $Y = 2·08x - 0·74$	2·76 ± 0·06
B. $Y = 2·08x + 0·50$	2·16 ± 0·10
C. $Y = 2·08x - 0·19$	2·50 ± 0·06

TABLE 6. *Comparison of toxicity of natural pyrethrins* (A), (±)-*allylrethronyl* (+)-trans-*chrysanthemate and* (B) (±)-*allylrethronyl* (±)-cis-trans-*chrysanthemate* (C) *against* Macrosiphum solanifolii Ashm., *potato aphid*, 21. xi. 49

	Dosage (% w/v)	No. of insects*	Mortality (%)	Corrected (% mortality)
A. 1	0·004	44	100	100
2	0·002	36	97	96
3	0·001	36	92	90
4	0·0005	37	57	45
5	0·00025	42	64	54
B. 8	0·008	35	97	96
9	0·004	37	70	62
10	0·003	40	63	53
11	0·002	41	46	31
12	0·001	40	33	14
C. 13	0·016	38	89	86
14	0·008	33	61	50
15	0·006	34	56	44
16	0·004	32	66	56
17	0·002	39	31	12
6+18	Sprayed control	106	22	—
7+19	Unsprayed	73		

Slope (b) = 2·36 ± 0·27.

The treatment difference is significant, see Fig. 11.

Spraying conditions: deposit 6·78 mg./cm.^{2}; temperature 20° C.; humidity 61·5 % R.H.

Storage of insects after spraying: temperature 18° C. (approximately); humidity 60 % R.H.

* These figures represent four replicates of about ten insects at each concentration.

The data could be fitted by three parallel lines.

Equations of probit lines	Log (LD 50 × 10^5)
A. $Y = 2·36x + 1·38$	1·53 ± 0·07
B. $Y = 2·36x - 0·75$	2·44 ± 0·05
C. $Y = 2·36x - 1·54$	2·77 ± 0·06

TABLE 7. *Comparison of toxicity of natural pyrethrins* (A), (±)-*allylrethronyl* (+)-trans-*chrysanthemate* (B) *and* (±)-*allylrethronyl* (±)-cis-trans-*chrysanthemate* (C) *against* Phaedon cochleariae *Fab., mustard beetle*, 6. xii. 49

		Dosage (% w/v)	No. of insects*	Mortality (%)
A.	1	0·004	30	97
	2	0·002	30	100
	3	0·001	30	77
	4	0·0005	29	72
	5	0·00025	28	46
B.	8	0·004	27	89
	9	0·002	30	80
	10	0·001	30	53
	11	0·0005	31	39
	12	0·00025	30	17
C.	13	0·016	30	97
	14	0·008	30	97
	15	0·004	20	85
	16	0·002	32	50
	17	0·001	26	31
	6+48	Sprayed control	59	0
	8+49	Unsprayed	48	0

Slope (b) = 2·03 ± 0·04.

The treatment difference is significant, see Fig. 12.

Spraying conditions: deposit 6·52 mg./cm.^{2}; temperature 19·5° C.; humidity 46·5 % R.H.

Storage of insects after spraying: temperature 17° C. (approximately); humidity 55 % R.H. (approximately).

* These figures represent three replicates of about ten insects at each concentration.

The data could be fitted by three parallel lines.

	Equation of probit lines	Log (LD 50 × 10^5)
A.	$Y = 2·03x + 1·93$	1·51 ± 0·08
B.	$Y = 2·03x + 1·13$	1·91 ± 0·08
C.	$Y = 2·03x + 0·52$	2·21 ± 0·07

TABLE 8. *Comparison of toxicity of natural pyrethrins* (A), (±)-*allylrethronyl* (+)-trans-*chrysanthemate* (B) *and* (±)-*allylrethronyl* (±)-cis-trans-*chrysanthemate* (C) *against* Oryzaephilus surinamensis *L., saw-toothed grain beetle*, 20. xii. 49

		Dosage (% w/v)	No. of insects*	Mortality (%)	Corrected (% mortality)
A.	1	0·04	65	98	98
	2	0·02	67	97	97
	3	0·01	63	75	74
	4	0·005	60	23	20
	5	0·0025	62	39	36
B.	8	0·08	66	98	98
	9	0·04	65	83	82
	10	0·02	64	61	59
	11	0·01	60	57	55
	12	0·005	65	34	31

Table 8 (*continued*)

C. 13	0·16	66	100	100
14	0·08	66	98	98
15	0·04	65	88	88
16	0·02	61	54	52
17	0·01	61	46	44
18+6	Sprayed control	129	4	—
19+7	Unsprayed	126		

Slope (b) = 2·15 ± 0·15.
The treatment difference of B and C is not significant, see Fig. 13.
Spraying conditions: deposit 6·79 mg./cm.²; temperature 16·5° C.; humidity 54% R.H.
Storage of insects after spraying: temperature 17° C.; humidity 55% R.H.
* These figures represent four replicates of about fifteen insects at each concentration.
The data could be fitted by three parallel lines.
The residual χ^2 is significant ($\chi^2 = 37·879$) and a heterogeneity factor has been used.

Equations of probit lines		Log (LD 50 × 10^5)
A.	$Y = 2·15x - 0·86$	2·73 ± 0·08
B.	$Y = 2·15x - 1·55$	3·05 ± 0·08
C.	$Y = 2·15x - 1·70$	3·12 ± 0·11

Discussion

From the evidence presented, it will be seen that the absolute toxicity of the materials based on the L.D. 50's varies considerably with the test-subject used. This is illustrated in Table 4. The last column gives an indication that, to the insects investigated, the synthetic materials are less specific than the natural pyrethrins, although, with one exception, also less toxic.

An inspection of Table 1 shows that the two synthetic esters were less toxic than the natural pyrethrins to three of the four test-subjects, and the comparison is particularly unfavourable with the aphid species used. However, with the caterpillars of *Plutella maculipennis*, both esters proved to be more toxic than the natural pyrethrins, the partially synthetic material being three to four times more effective on a weight basis when evaluated as described.

The results which are set out in Tables 2 and 3 leave little doubt that the relative toxicity of these closely related compounds varies considerably with the different insect test-subjects, the extremes for the comparison of the partially synthetic material with the natural pyrethrins being from about one-eighth as toxic with *Macrosiphum solanifolii* as test-subjects to nearly four times as toxic with *Plutella maculipennis*. For the fully synthetic material it ranges from approximately one-sixteenth as toxic as the natural pyrethrins with *Macrosiphum solanifolili* as test-subjects to nearly twice as toxic with *Plutella maculipennis*. The partially synthetic ester was almost exactly twice as toxic as the fully synthetic one to *Plutella maculipennis* larvae, to *Macrosiphum solanifolii* and to *Phaedon cochleariae*. However, the completely synthetic material was considerably more than half as toxic as the partially synthetic one to *Oryzaephilus surinamensis*.

These variations in relative, as apart from absolute, toxicity seem notable considering

that the esters used varied only in details of their structure and in their stereochemistry. Since the natural pyrethrins are a mixture of at least four esters from two keto alcohols and two acids, they leave much to be desired as a valid standard of comparison, as has been pointed out by Gersdorff (1949*b*). It has so far been impossible to synthesize all of the four esters as they occur naturally or to separate them in a pure state from pyrethrum concentrates. The difficulty, therefore, is to know to what extent the relative toxicity of the active natural components of the standard is varying with the test-subject. It may be that differences in physical properties (especially between the esters of chrysanthemum monocarboxylic acid and those of chrysanthemum dicarboxylic acid monomethyl ester), acting in conjunction with possible differences in intrinsic toxicity (i.e. the interference with the insect metabolism when the compounds have reached their site of action) may account, at least to some extent, for the variable ratios found between this standard mixture and the two synthetic materials, when used on a number of different insect species.

The factors affecting the relative toxicities of the two synthesized esters might be expected to be less complex than those involved in comparisons between the natural mixture of four esters and the two synthetic compounds. The allyl keto-alcohol is common to both materials and the *dextro* rotatory *trans* chrysanthemum monocarboxylic component of the partially synthetic ester is present in the racemic *cis-trans* (synthetic) chrysanthemum monocarboxylic acid portion of the completely synthetic ester. Gersdorff (1949*a*) deduced that the *dextro* rotatory forms of the *cis* and *trans* chrysanthemum monocarboxylic acids (but not the *laevo* ones) were effective when esterified with the cyclic keto-alcohols. This is in line with the results from the three insect species to which the ester from the (+)-*trans* acid was twice as toxic as that from the (±)-*cis-trans* form (Table 2, *Plutella maculipennis*, *Phaedon cochleariae* and *Macrosiphum solanifolii*). In this case a toxic compound is being compared with a mixture of itself diluted with less toxic isomers, in contrast to the comparisons referred to earlier between the four natural esters, all of high activity, and the two synthetic materials. On the other hand, the completely synthetic material was considerably more than half as toxic as the partially synthetic one to *Oryzaephilus surinamensis* (Table 2), and without further investigation of these results, any explanation put forward must be speculative. However, the possibility cannot be dismissed that some components of these mixtures investigated are active against some species of insects and not against others, or that they show some synergistic activity with some species and not with others (when their toxicity is evaluated by the method described). It may be significant that these variations in relative toxicity to various species of insects only occur when the natural pyrethrins are compared with either synthetic material, and that such wide variations have not, as yet, been observed in comparisons of the toxicity of the partially synthetic and completely synthetic esters.

The fact that insecticides may differ in their relative toxicity according to the species used as test-subject (Busvine, 1942; Müller, 1946; Martin, 1948) is now

generally accepted, and Beard (1949) has shown that this phenomenon occurs even when the chemicals are administered externally, orally or by injection and then ranked on the results by the most effective route. A study of the literature, however, indicates that recognition of this specificity of effect is frequently limited to compounds of widely differing structure, while the position with more closely analogous chemicals has not been defined. Lord (1948) observed a variation in relative toxicity with species amongst various D.D.T. analogues, and it must be pointed out that such differences in the relative potency of structurally related chemical compounds according to the test-subject used, are added complications to the study of the relationship between molecular structure and configuration and insecticidal activity. Busvine (1942) gives data on the toxicity of fat solvent vapours which he interprets as showing that with poisons which he states to be mainly physical in their action, the relative toxicity remains constant irrespective of the species used as test-subject, but it is not easy to define the mechanism of action of any given poison.

The evidence seems to be sufficient to show that specificity of effect must be considered when studying the relationship between constitution and toxicity even when the chemicals are of such similar structure that they may be expected to be acting on the same process in the insect's metabolism.

As far as we are aware this point has not been very clearly stated, and in the past examinations of the relationship between the structure and toxicity of a closely related series of compounds have been carried out on a single test-species, which must limit the significance of the results obtained, in the absence of more detailed knowledge of the causes of specificity. Picard & Kearns (1949) have drawn attention to this fact in connexion with a study of D.D.T. and closely related compounds, but their statement is based on work where two different bioassay techniques were used and it is possible that the difference in testing procedure may have affected the results. It is not regarded as impossible that changes which affect toxicity can be made in the molecular structure of closely related chemicals which would give a constant ratio of toxicity when the substances were tested on a number of species of insects, but it is apparent from these results that there are limitations which are not yet defined to the alterations having this effect.

Leaving aside the more theoretical considerations, the overall evidence is that the synthetic compounds examined are most promising in their insecticidal activity. Until further information is available on their physical and chemical properties such as stability and on the cost of production, it would not appear possible to assess their practical possibilities, but the results do show that materials of high biological activity allied to the pyrethrins can be synthesized and make the prospect of obtaining a satisfactory commercial product more likely.

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BIOASSAY SYSTEMS FOR THE PYRETHRINS

III. APPLICATION OF THE TWIN CROSS-OVER DESIGN TO CRAWLING INSECT ASSAYS

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The special properties of pyrethrum assays with crawling insects by the film technique lend themselves to the twin cross-over design, hitherto restricted to assays with graded responses. The conditions under which this design is appropriate, and the treatment of control responses, are discussed. The design is particularly efficient with laboratory cultures of insects with fairly stable tolerance levels, and is also useful with heterogeneous populations collected in the field. Examples of the increased precision afforded are considered. The reversibility of the primary responses of grain weevils to the pyrethrins is also demonstrated.

Introduction

A number of experimental designs, intended mainly for the rabbit assay of insulin by determination of blood sugar, have been devised, and an efficient and convenient method known as the twin cross-over design, embodying the advantages of the earlier designs has been described by Smith, Marks, Fieller & Broom (1944).

In this design two dosage levels of the standard and test solutions are used, both solutions and dosage levels being 'crossed over' between the occasions of testing. Groups of test organisms receiving, say, a high dose of standard solution on the first occasion would, after recovery, be given a low dose of the test solution. The other three groups, initially receiving the low standard, high and low test doses would similarly be submitted to the high test, low and high standard doses respectively.

The test organisms are thus used twice, the doses applied on each occasion being allocated so that the error variance in the subsequent analysis of the data is based on the tolerance difference of a given batch of organisms between occasions of testing, rather than on the variation between batches on any one occasion. In the insulin assay of Smith *et al.* this innovation leads to an increase of precision, since 'batches of one' are there used with graded response.

Heubner & Stühlmann (1940) have applied an earlier cross-over design for an assay of oestrone which includes the use of quantal responses, but hitherto such techniques have not apparently been adapted for insecticidal assays.

Where applicable, the twin cross-over design appears of value in cases where the test-insects can only be obtained in small homogeneous batches, such as can be reared from collected egg batches, aphis colonies on leaves, and insects collected in the field generally.

An increase in precision will also result from the adoption of the principles of

this design whenever the temporal fluctuations in the tolerance of the test organisms during the period of testing are less than the heterogeneity between batches. Such an increase was shown for less efficient cross-over designs by Heubner & Stühlmann (1940).

For fumigants, stomach poisons, and for contact insecticides such as D.D.T. and benzene hexachloride, which for most purposes can be assayed only by such responses as mortality or irreversible paralysis, the test-insects can be used once only. With the pyrethrins, however, the primary responses of activation and paralysis which are completely reversible are available, and the advantages of using such responses in biological assays have been outlined by Page, Stringer & Blackith (1949).

If all the batches of test-insects can be drawn from a homogeneous population, as with many laboratory cultures, a tolerance level caused to fluctuate by the first set of treatments could lead to a reduced recovery of information as compared with the conventional type of assay, a situation which occasionally arises in factorial experiments with an inappropriately chosen system of 'blocks'. The use of a twin cross-over design cannot, however, cause this information to be lost, since Fieller (1944) has demonstrated that the data from any such assay can be alternatively analysed by the usual methods for replicated relative potency assays (Finney, 1947; Emmens, 1948). Sources of variation due to doses, samples, temporal variation, and error may then be segregated and compared.

A double analysis on these lines, though informative, would be tedious as a routine measure, so that a preliminary experiment was designed to examine the effect of varying doses of the pyrethrins on 2–3-week-old adult grain weevils, *Calandra granaria* L., in terms of the resistance of the insects to a second dose of the insecticide, when they had been allowed to recover from the first.

Methods

Twenty-four batches of thirty insects, all drawn from a homogeneous population, were starved for 12 hr. in ventilated specimen tubes held at 25° C. The insects were standardized by the method of Stringer (1949). Three sets of eight 7 cm. no. 1 Whatman filter-papers were impregnated with 0·1 ml. of heavy white oil containing respectively 0·40, 0·20 and 0 % pyrethrins. This extract was obtained by dilution of the 20·3 % extract, M 207, kindly provided by Mr R. P. Merritt of Stafford Allen and Sons Ltd. Even distribution of the oil over the filter-paper was secured by diluting the oil with four times its volume of 'Analar' petroleum ether (boiling range 100–120° C.) 'freed from aromatics'. This additional solvent, which facilitates handling, reduces drainage errors, and ensures rapid flooding of the substrate, is allowed completely to evaporate before the introduction of the insects. The development and properties of this rapid method of application will be described in detail elsewhere.

The insects were confined on the impregnated papers by 6·5 cm. diameter glass

rings for 12 hr. at 30° C., and the proportion paralysed at the end of the exposure period counted. A modification of the warm-plate method of stimulating the insects before counting was used (Tattersfield & Potter, 1943) in which a 15 W. 'Anglepoise' lamp was placed for 2 min. about 3 in. above the insects, giving a surface temperature (black-body absorption) of 38–40° C. The insects were classified as 'paralysed' and 'not paralysed', since finer subdivisions of the response do not, in practice, seem to give much additional information (Finney, 1947). The mean level of response with 0·40 % pyrethrins was 76·1 ± 7·3 %, and that for 0·20 % was 42·4 ± 14·8 %. There were no insects affected amongst those confined on filter-papers treated with the oil carrier only.

All the insects of each batch were transferred, after classification, to clean wheat in separate specimen tubes, and allowed to recover for 24 hr. at 30° C. At the end of the recovery period, the insects were sorted off and starved as before.

The three samples of insects pre-treated respectively with the high and low doses of the pyrethrins, and the oil carrier only, were then submitted to a further experiment designed to detect any differential change in their tolerances. Each sample was divided into four sets of two replicated groups of thirty insects, which were confined on filter-papers impregnated with four sets of concentrations of the pyrethrins equally spaced on a logarithmic scale. The proportions paralysed at the end of 12 hr. are recorded in angular units in Table 1.

TABLE 1. *Showing proportions of insects paralysed after* 12 hr.

	Pyrethrins concentration (%)			
	0·17	0·25	0·36	0·52
Sample pre-treated with:				
(A) Oil carrier only	21·0	33·0	49·0	50·8
	21·4	31·1	39·2	60·0
(B) 0·2 % pyrethrins	27·9	33·0	43·1	56·0
	18·4	29·4	25·5	56·9
(C) 0·4 % pyrethrins	19·5	26·6	35·3	56·9
	23·7	27·9	36·7	52·8

DISCUSSION

Analysis of variance of the data of Table 1 shows that there is no significant difference between the tolerances of the two treated samples when compared with that subjected to the oil carrier only. The probit regression lines obtained from the three samples were examined for significant departures from linearity and parallelism, which were not found.

These facts suggest that the paralysis of *C. granaria* by the pyrethrins is completely reversible, and involves no permanent effect on the tolerances of the treated insects.

Insects paralysed by the pyrethrins are capable of mating and laying viable eggs

fertilized prior to treatment in the grain on which they spend the recovery period within 12 hr. of exposure to the insecticide.

The possibility was also considered that the pre-treatment with the insecticide would set up an interaction between the applied dose and the variance of the responses, thus rendering less certain the conclusions drawn from the analysis of variance. Bartlett's test, which may validly be applied to the data of Table 1 after elimination of the linear regression (Bishop & Nair, 1939), shows that this situation does not arise. Thus the use of a twin cross-over design will ordinarily result in an increase of precision in assays involving the system outlined above, since the temporal variation over the periods considered of the tolerances of stocks of *C. granaria* is usually less than their sampling variance.

TABLE 2. *Allocation of insects to doses in estimation of relative potency by the twin cross-over design*

(Standard solutions: high dose, 0·4% pyrethrins; low dose, 0·2% pyrethrins. The corresponding high and low doses of the test solutions, in this illustrative example, are diluted 10 times relative to the standards, and contain a proportion of the pyrethrum synergist, piperonyl butoxide, 8·5 times that of the pyrethrins.)

Groups	Replicates	Responses on First day	Responses on Second day
		Standard high	Test low
1	1	60·7	22·0
	2	57·4	21·1
	3	57·4	16·4
		Standard low	Test high
2	1	25·1	33·8
	2	30·7	46·7
	3	29·3	40·4
		Test high	Standard low
3	1	35·7	29·3
	2	35·1	28·0
	3	43·3	34·4
		Test low	Standard high
4	1	12·9	62·0
	2	17·5	49·0
	3	23·6	49·6

The relative potency of the two preparations, together with its fiducial limits, is included in Table 3.

DESIGN AND ANALYSIS OF TWIN CROSS-OVER EXPERIMENTS

The layout of treatments for a typical assay is given in Table 2. The responses there shown are the angular transforms of the percentages of weevils paralysed. The statistical analysis may conveniently be carried out in terms of the sums and differences of these quantities, and of their reduced sums of squares. The details are described by Smith *et al.* (1944) and will not be repeated here. The theoretical

basis of the design is the subject of a further paper by Fieller (1944). In calculating the fiducial limits for pyrethrum assays, the value of t used by Smith *et al.* may be replaced by the corresponding deviate derived from the normal distribution, since these assays involve a quantal response.

The treatment of control responses also requires consideration. At first sight, the situation might appear complicated. Up to the end of the first treatment period, control responses will commonly be estimated by separate batches of insects placed on oil carrier only. The usual assumption that control responses are uncorrelated, or only slightly correlated, with the treatment responses is probably adequate here, as in most similar cases, despite the criticism of Horsfall (1945). The presence of marked correlation between the two types or response will cause the adjustments developed by Finney (1944) to be slightly in excess of those required by more detailed considerations, but the effect is in any case small. The matter has been further discussed by Finney in a recent paper (1949).

A further set of responses will then be superimposed on the first, consisting of a few cases of irreversible paralysis caused by the initial set of treatments, and leading to the death of the insects concerned. These responses could be estimated by running duplicates of the original controls through the first set of treatments, but in practice the necessity for this elaboration does not arise. This second set of control responses will be very highly correlated with the treatment responses, and it is most unlikely that any experiment of practicable size would distinguish a value for the correlation coefficient significantly less than unity. In addition, with the short exposure periods appropriate to assays using primary responses, the values of either kind of control responses will be very low. In the experiment detailed above, the control responses for the 720 insects involved were: Type 1, 0; Type 2, 3. Such figures appear to be representative of other assays conducted by the author using the twin cross-over design. If the dosage range for the first test is misjudged, however, so that the primary responses are high, the secondary response of mortality may become appreciable.

Since the control responses of the second kind will usually be both small and highly correlated with the treatment responses, the correction to be applied to the data from the second occasion of testing will be negligibly small. This situation arises from the fact that as the regression lines for the primary and secondary responses have different slopes, the estimation of the resultant response corresponds *mutatis mutandi* with the estimation of that produced by a mixture of two poisons having 'correlated independent action', discussed in some detail by Finney (1947). Unless the first set of dosages has been so misjudged as to produce very high primary responses the resultant secondary responses will follow those expected for the treatments on the second occasion of testing almost indistinguishably, as Finney was able to show for the similar case of mixed toxicants. Any insects dying in the period between the first and second paralysis counts may thus be included as 'paralysed' in the second count.

Efficiency of the experimental design

An obvious economy of experimental material is effected when the test-insects are used on two occasions, but shortage of materials from laboratory cultures rarely limits the size of experiments. On the other hand, such cultures are generally maintained under standardized conditions and are frequently of species, such as *C. granaria*, which have a moderately stable level of tolerance over a period of a few days. As indicated earlier, whatever the relative magnitude of the temporal and sampling variance may turn out to be, the data from any twin cross-over experiment can be alternatively analysed so as to base the estimate of error on the sampling, rather than the temporal, variance. Similarly, should some mishap occur to the experiment on the second occasion of testing, the data for the first day's test may be analysed as a 'four-point' assay, though with less than half the information obtainable from the completed experiment.

These alternative methods of analysis may be made the basis of an estimate of the efficiency of the new design, since the amount of information contributed by an observation is inversely proportional to its variance. A very close approximation to the best estimate is provided by the square of the ratio of the fiducial range of the estimate of relative potency from the twin cross-over analysis, to that obtained from an analysis of the data as a 'four-point' assay.

The double analysis necessary has been done on a number of assays of the relative potencies of mixtures of the pyrethrins and piperonyl butoxide, compared with the pyrethrins alone. These assays were conducted by a technique essentially similar to that outlined earlier in this paper, using a twin cross-over design with three replicates per group, tested against two doses each of the standard and test solutions. In order to obtain solutions of roughly equal potency, it was necessary to dilute the mixtures of pyrethrins and synergist. In certain experiments, notably those with synergist ratios of 3, 4 and 5 to 1 where the dilution factor was underestimated, the responses to the mixture were high, and the precision of the assays low. In these circumstances the twin cross-over design appears exceptionally effective in salvaging information, although the inherent precision remains lower than in the well-balanced experiments.

Table 3 gives the results of a series of assays in which the efficiency of the twin cross-over design has been estimated, relative to that provided by an analysis of the same data using the sampling variance as error.

As will be observed from Table 3, a considerable increase in the amount of information recovered from an individual assay is made possible by the use of this design, which involves little more work than laying out an experiment on the usual factorial lines. The analysis is rather longer than the short computation for assays with equally spaced log doses outlined by Emmens (1948), and is comparable in length with the more general method offered for the factorial design by Finney (1947).

TABLE 3. *Relative potencies of some mixtures of piperonyl butoxide and the pyrethrins*

Mol. ratio of synergist	Dilution factor	Log ratio of potency	Relative potency	95 % fiducial range log scale		Efficiency factor	95 % fiducial limits, Linear scale			
							X-over		4-point	
				X-over	4-point		Lower	Upper	Lower	Upper
0·25	1/1	0·291	1·96	0·400	0·801	4·00	1·64	2·47	0·64	5·93
0·50	1/1	0·414	2·59	0·200	0·228	1·31	2·06	3·26	2·00	3·37
0·75	1/1	0·332	2·15	0·138	0·860	38·8	1·86	2·56	0·79	5·78
1·0	1/1	0·478	2·95	0·151	0·170	1·26	2·52	3·46	2·43	3·59
1·5	1/1	0·541	3·48	0·550	1·551	8·00	1·94	6·24	0·58	20·7
2·0	1/1	0·635	4·31	0·307	0·937	9·30	3·25	6·57	1·48	13·62
3·0	1/1	0·746	5·58	0·560	11·82	446·0	2·93	10·63	$1·98 \times 10^{-5}$	$1·57 \times 10^{6}$
4·0	1/2	0·892	7·79	0·510	3·48	46·6	5·23	16·83	0·142	429·0
5·0	1/2	0·945	8·81	0·430	5·40	153·5	5·88	13·21	0·0195	4900·0
6·0	1/10	0·915	8·22	0·128	0·604	22·2	7·09	9·52	4·33	15·65
6·5	1/10	0·795	6·23	0·128	0·420	10·8	5·35	7·26	4·01	9·71
7·5	1/10	0·710	5·14	0·177	0·838	22·0	4·18	6·30	2·13	12·33
8·5	1/10	0·780	6·03	0·104	0·548	27·8	5·35	6·80	3·77	11·97
9·0	1/10	0·770	5·89	0·0470	0·289	37·9	5·60	6·20	4·34	8·00
10·0	1/10	0·773	5·93	0·0452	0·460	102·2	5·58	6·24	3·66	9·62
11·0	1/10	0·678	4·77	0·208	0·675	10·24	3·75	6·06	2·33	9·73
12·0	1/10	0·676	4·75	0·216	0·391	3·27	3·72	6·04	3·12	7·22

The twin cross-over design is not suited, in the form used by Smith *et al.* (1944) and in this paper, for the investigation of dosage-response relationships, since only two doses of the insecticide are used. As Emmens (1948) indicates, more dosages could be introduced at the expense of enlarging the experiment, by increasing the number of occasions of testing, but this type of assay and its statistical analysis would be rather cumbersome for routine work. The design is particularly adapted to routine estimates of relative potency with the pyrethrins using crawling insects or flying insects confined to a surface, and for this purpose its use seems to offer considerable advantages over the customary designs. Several estimates of the same relative potency may be pooled, by the method given by Fieller (1944), if higher precision is required than that afforded by a single test.

The variance of estimates of relative potency by this method compares favourably with those obtained by chemical analysis. A quantitative comparison with the latter method would, however, involve differences of opinion on the chemical assays which cannot be considered here.

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FIELD METHOD FOR THE DETERMINATION OF THE PARTICLE SIZE OF OIL MISTS

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(With Plate 14 and 5 Text-figures)

For the control of plant pests, e.g. insects, spraying machines have of late been developed, especially in the U.S.A., with which low dosages of fairly concentrated solutions of toxicants in mineral oils are sprayed. The efficiency of such products is no doubt influenced by the fineness of the spray. An accurate method of determining this fineness was desirable both for the control of the spraying operation and for evaluation of the merits of spraying apparatus.

Knowledge of the average drop size is inadequate in this respect; it is necessary to know the distribution of the oil in droplets of different sizes. A reliable impression of this percentage can be obtained only if a large number of particles are measured. These measurements can be used to plot frequency curves based on number or volume distribution.

A simple microphotographic method is described whereby these measurements can be made both in the laboratory and in the field. The procedure makes it possible to obtain, shortly after spraying, a good idea of the size of the oil drops in the deposit.

An apparatus was designed and constructed to determine the diameter frequency curve and the volume distribution curve of oil droplets deposited in field spraying on coated glass plates.

The method gave characteristic results for field sprayings with the Strawsonizer and a helicopter fitted with a spraying boom and also in the laboratory when using a spraying apparatus with different arrangements of the nozzle.

Introduction

In combating plant pests mineral oils were, until recently, generally sprayed in the form of dilute emulsions of oil in water. The present trend, however, especially since the development of new types of spraying machines in the U.S.A., is to use smaller volumes, i.e. higher concentrations of the toxicants, often in the form of straight solutions in oil. This development opens up an entirely new field for the use of spray oils.

A primary requirement of such sprayings is that the oil and its toxicants are distributed over the crop and the parasites with sufficient homogeneity.

In case the toxicant retains its activity for some time (residual effect) and the insects are mobile, or if oil spreads spontaneously over the whole surface after application, the total amount of spray liquid is of primary importance: when this amount is sufficient all parasites come into contact with a toxic quantity of the spray.

When applying non-spreading sprays or contact sprays without residual effect, however, the insect or the egg should be contacted immediately with enough spray

to render the control effective. To reduce the part of the surface not covered by spraying a given volume of such liquids, the droplets should theoretically be as small as possible, but when the mist becomes finer the risks of loss of spray by wind drift and the formation of stable non-depositing mists increase. Static electricity on surface and droplets may counteract deposition of fine droplets; during spraying experiments in the United States it has been noticed that only a small percentage of droplets smaller than 5μ is deposited on foliage or glass plates.

What particle size is most effective will depend on several conditions, such as the size of the parasites to be controlled and the technique of spraying. When aircraft is used the average size of the droplets must not be too small otherwise the mist will be dispersed by the wind.

For an adequate study of such problems it is necessary to evaluate the size of the mist particles in the field. The results thus obtained can be represented in a frequency curve showing the distribution of the *number* of particles over the various size classes. It is also important to gain information about the distribution of the *volume* of the oil over the particles of the various size classes. Such a 'volume distribution curve' can be easily derived from the frequency curve of the particle size.

Such curves can only be determined with sufficient accuracy if a large number of particles is taken into account. Measurements by direct microscopy take too much time and are difficult to make in the field. Therefore we developed an assembly by means of which frequency curves of the particle size in the field and in the laboratory can be quickly determined. The method consists in collecting the droplets on coated glass plates on which the atomized liquid does not spread, and photographing sections of the deposit. The size of the particles is determined in much enlarged projections of the photographs.

Method of measurement

Two methods are described in the literature (Anderson, 1948; May, 1945) for the preparation of surfaces on which oil drops can be collected and measured. In one method the glass is coated with a thin film of a material, such as mannitan monolaurate, octadecylamine (Armeen 18D), organo-silicons (Drifilm 9987). On surfaces of this kind the oil forms lens-shaped spots that do not coalesce. The dimensions of these spots can be measured microscopically. As stated above, particles smaller than about 5μ will not be deposited quantitatively. In the other method the glass surface is coated with a layer of a fluffy non-coherent powder such as soot, magnesium oxide or aluminium oxide. When a drop falls with a certain momentum on this layer a crater is formed, the diameter of which can be determined microscopically. This method is especially recommended for spray liquids of high volatility, because if the former method is used, partial evaporation may occur before the determination of the particle size is completed. A drawback to the second method is that small particles do not form visible craters. Moreover, the size of the particles can only be determined approximately from the craters they form.

As it was desirable to include in our measurements the smallest particles that will settle on the plates, we used the former method, using glass coated with Drifilm to collect the droplets of oil. As soon as the droplets settled on the glass plates a number of microphotographs were taken; as this is a rapid method, it could be used for fairly volatile spray liquids such as kerosene.

The microphotographic installation consists of a Leitz microscope on which a 35 mm. film camera (Leica without a lens) is mounted, with the intermediary of a Leitz Micro Ibso attachment containing a double prism, so that the object to be photographed can also be observed visually (Morgan & Lester, 1947). When the microscope tube is placed so that the object is sharply observed by the eye, the picture on the film in the camera is also sharp. An iris shutter is fitted over the prism (for instantaneous and time exposures). The usual microscope objectives are used so that various enlargements can be applied (from *c.* 3·3 to 260 times). For our measurements we used enlargements of 3·3 to 35 times.

Illumination of the object was effected by means of a lamp (6 V., 6 W.) with a collector lens.

The photographs were taken on an orthochromatic Duplo film from Gevaert (Oude God, Belgium).

As it was often desirable to obtain, immediately after spraying, some idea of the particle size distribution, a box was constructed by means of which exposed films could be removed from the camera in the field, introduced into the developing tank and developed and fixed on the spot. After drying the pictures were projected.

The projector is constructed for use in a lorry. It consists of a brass tube, at one end of which a motor-car lamp* is mounted (6 V., 25 W.). By means of a double condenser the negative is evenly illuminated. The negative is passed through the projector tube through slits. The picture to be projected can be adjusted by hand into its proper place. The projection lens is a Leitz Elmar with focal length of 5 cm. and lens opening of 3·5. The projector is fixed vertically on a stand. The picture is projected on to a horizontal screen on a table; it can be easily measured with scale divisions applied on transparent material on which the corrected diameters of the drops are indicated for different enlargements. In this way 500 oil spots can easily be classified in 1 hr.

Field measurements are meant to afford a survey of the fineness of the deposit on the plants. For this purpose the plates must always be distributed at random over the sprayed area. In order to find out how the size of the spray droplets is distributed over the various parts of the field, the glass plates would have to be placed in special spots in the field. In field tests on agricultural crops the plates were fixed horizontally on iron stands so as to bring them nearly on a level with the tops of the crops to be sprayed.

* The lamps are of the 6 V. type, so that they can be connected to a motor-car accumulator battery, which enables the apparatus to be used in the field. At the laboratory the lamps are fed using an adequate transformer.

If the measurements are made with a view to obtaining a picture of the dispersion of a liquid by a certain nozzle the glass plates should be arranged so as to obtain systematically an average distribution.

Method of calculation

The diameter ($=2r$) of the spherical air-borne droplet can be calculated from the diameter of the spot on the glass plate ($=2A$) by means of a correction factor, r/A, which is determined by allowing an oil spot under the microscope to act as a plane-convex lens (May, 1945).

The focal length of the oil lens is determined by first focusing the microscope on the circumference of the spot and then by readjusting in such a way that the image of a distant object, formed by the oil lens,* is observed sharply. The distance which the microscope tube is shifted between the two adjustments (f') is read on the micrometer screw. The correction factor can be derived with the aid of Text-fig. 1. For this purpose a line is drawn from the abscissa value of $f'/2A$ parallel to the ordinate axis. The value of the ordinate belonging to the point of intersection with the curve of bundle I indicates the contact angle (θ) in degrees. A line parallel to the abscissa is drawn from this point of intersection; its point of intersection with curve II gives the reading for r/A on the top abscissa. Thus also $2r$, which represents the drop size, is known.

The curves of bundle I in Text-fig. 1 are based on the following equation

$$\frac{f'}{2A} = \frac{1}{2(n_D - 1)\sin\theta} + \tfrac{1}{2}\tan\tfrac{1}{2}\theta,$$

θ being the contact angle of the oil spot. These curves have been drawn for various values of the refractive index n_D of the oil.

Curve II is determined by

$$\frac{r}{A} = \frac{1}{\sqrt[3]{2}}\left[1 - (1 + \tfrac{1}{2}\sin^2\theta)\sqrt{(1 - \sin^2\theta)}\right]^{\frac{1}{3}} \operatorname{cosec}\theta.$$

The agreement found when calculating and measuring the contact angle showed that these equations may be applied in the cases investigated by us.

The correction factor thus determined (*c.* 0·4) was in satisfactory agreement with the values recorded by May (1945).

The number of drops needed for the measurements depends on the accuracy with which each of the figures (on which the frequency curve is based) is to be determined. To restrict the standard deviation for each size class to 10% of its value, it is necessary to count at least about 100 particles in the size class in question. If a p-times smaller deviation is required the number of particles to be counted will have to be p^2 times as large.

* In this case the flat mirror of the microscope is used, the condenser being removed.

This statement is based on the following calculation:

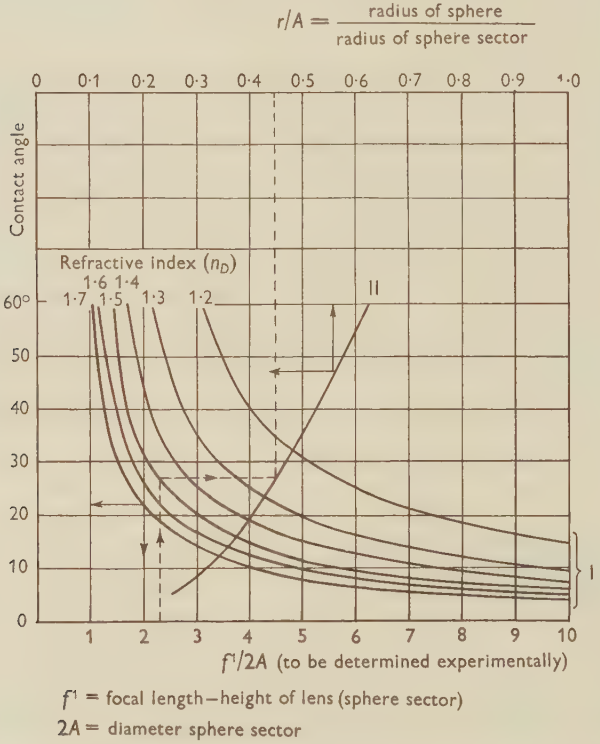
If n = the total number of particles counted,

n_i = the number of particles counted in the size class i,

$h_i = n_i/n$ the fraction of the particles in the size class i;

then the standard deviation S_{n_i} of the number of particles n_i may be calculated from

$$S_{n_i} = \sqrt{[h_i(1-h_i)n]}. \qquad (1)$$



Text-fig. 1. Graph to determine the factor by means of which the radius of the spherical oil drop can be found from the radius of an oil spot with the same contents.

The standard deviation S_{h_i} of the fraction h_i is equal to

$$\frac{Sn_i}{n}, \quad \text{so} \quad S_{h_i} = \sqrt{\frac{h_i(1-h_i)}{n}}. \qquad (1a)$$

It should be emphasized that equations (1) and (1 a) are approximate formulae, which give good results only if h_i is not too small.

From equation (1) it follows that

$$S^2_{n_i} = n_i(1-h_i).$$

In case we want the relative standard deviation to have a value of at most 10 % ($S_{ni} \leqslant 0{\cdot}1 n_i$) it follows that

$$n_i(1-h_i) \leqslant (0{\cdot}1 n_i)^2, \tag{2}$$

$$n_i \geqslant 100(1-h_i).$$

Therefore it is sufficient to count at least 100 particles in the size class i. It appears in general that if a p-times smaller deviation is required the number of particles to be counted will have to be p^2 times as large.

After a sufficient number of spots had been measured, and the above correction for the diameter had been applied, frequency curves of the particle size were plotted, the *number* of drops in each size class being indicated as a percentage of the total number measured.

From the corrected diameter the volume of the drops was calculated and the percentage which the summed *volume* of the drops belonging to one size class of oil drops was determined. This 'volume distribution curve' shows how the volume of the oil deposited is distributed over the various classes of particle size. Naturally, in this curve occasional large drops are of much greater importance than a great number of very fine droplets, the total volume of which constitutes only a small fraction of the entire volume.

It has been found that for large particles the size classes contain only a few particles. In this region, therefore, the distribution curve is very inaccurate. To increase the accuracy there is, however, the possibility of combining the size classes situated at the extreme end of the distribution curve into one class which then comprises a sufficient number of particles. It is impossible, however, to determine the total volume of the particles in such a large class, as the distribution of the volumes of the individual particles in this wide range is unknown.

In this case the following statistical method may be used.

The total volume V_k of the particles in all classes beyond a certain size is determined by adding their individual volumes

$$V_k = \sum_{i>k} n_i v_i, \tag{3}$$

where v_i = the volume of one particle in size class i and the summation is extended over all classes $i > k$.

The standard deviation of V_k is related to the standard deviations of the number of particles of the different size classes (n_i) in the following manner:

$$S_{V_k}^2 = \sum_{i>k} v_i^2 S_{ni}^2. \tag{4}$$

In this equation S_{ni} may be substituted from equation (1). As has been stated before, the application of equation (1) to classes containing only a small number of particles leads to inaccurate results for S_{ni}. However, in carrying out the summation in equation (4) these inaccuracies are levelled out to a large extent.

By substitution, the following formula is obtained, which will give sufficiently accurate results:

$$S^2_{V_k} = \sum_{i>k} v_i^2 h_i (1-h_i) n = \sum_{i>k} \frac{(v_i n_i)^2}{n_i} (1-h_i).$$

When h_i is small $(1-h_i)$ may be taken $=1$, so

$$S^2_{V_k} = \sum_{i>k} \frac{(v_i n_i)^2}{n_i}, \tag{5}$$

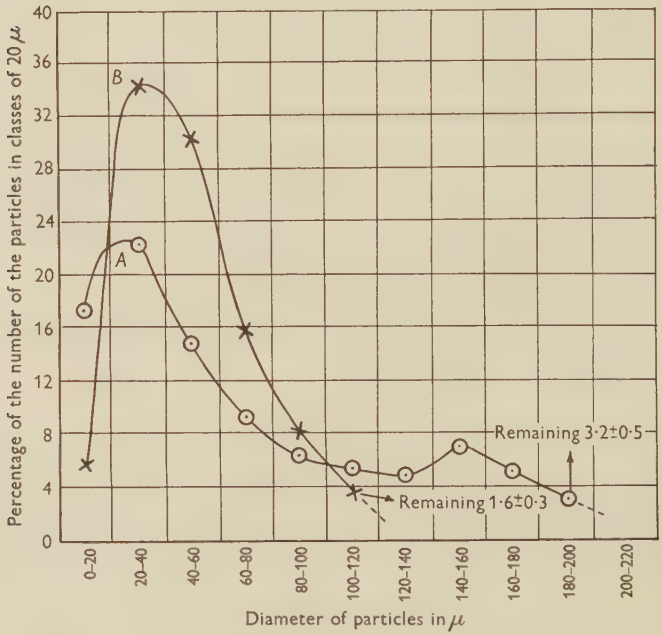
where $v_i n_i$ is the total volume of all particles in a size class.

The appended graphs also give the *fraction* of the volume $(=R_k)$ of the particles in all classes beyond a certain size:

$$R_k = \frac{V_k}{V} \text{ if } V \text{ is the summed volume of all particles counted.}$$

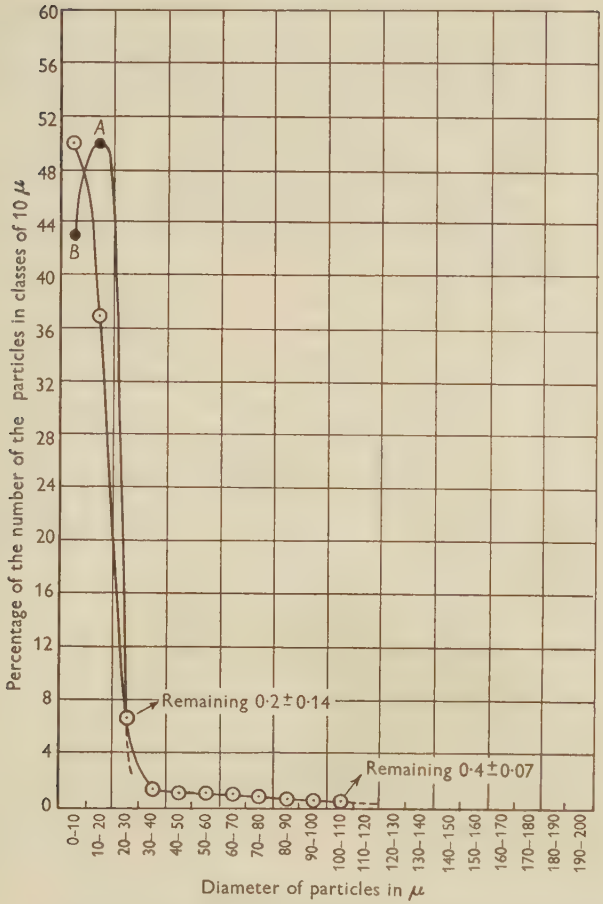
The standard deviation of $R_k\,(=S_{R_k})$ is obtained by dividing S_{V_k} by V, so:

$$S^2_{R_k} = \sum_{i>k} \frac{(v_i n_i)^2}{V^2} \frac{1}{n_i}. \tag{5a}$$



Text-fig. 2. Number-frequency curves of droplet sizes produced by technical spraying machines.

Spraying machine	Liquid sprayed
A, Helicopter	Light mineral oil
B, Strawsonizer	Light mineral oil



Text-fig. 3. Number-frequency curves of droplet sizes produced by laboratory spraying equipment. *A*, normal adjustment for laboratory investigation; *B*, coarsest possible adjustment. Liquid sprayed: light mineral oil.

Results

The above-mentioned method was applied to field sprayings with a Strawsonizer* and with a helicopter† and in the laboratory in the test apparatus described by Ten Houten & Kraak (1949).

* Atomizer from the Strawson Chemical Co. Ltd. London, provided with five spray nozzles each with nine orifices (diameter of each orifice 0·6–0·8 mm.). Spraying pressures *c.* 3·5 kg./cm.^{2} (air pressure).

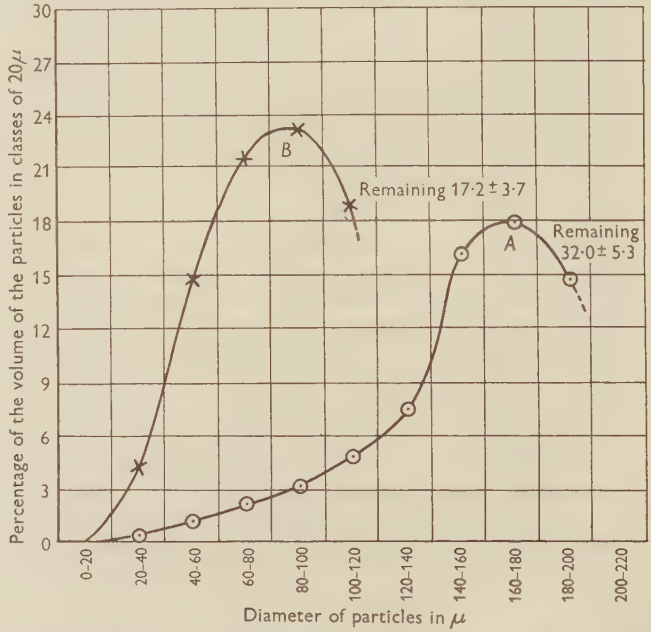
† Fitted with a spraying boom with eight Lyunet atomizing nozzles (orifice diameter 3 mm.). Liquid pressure about 9 kg./cm.^{2}.

(1) With the apparatus adjusted as it has been used in laboratory experiments so far (curve *A* in Text-fig. 3).

(2) With this apparatus adjusted to form the coarsest possible mist (curve *B*). In this case, however, the distribution of the drops on the surface sprayed was irregular.

In all these experiments a light mineral oil was sprayed. The results are given in Text-figs. 2 and 3 which show the number frequency curves of the particle size.

Oil spots from these different mists, collected on glass plates coated with Drifilm are shown in Pl. 14, figs. 1–3.



Text-fig. 4. Volume distribution of spray droplets produced by technical spraying machines.

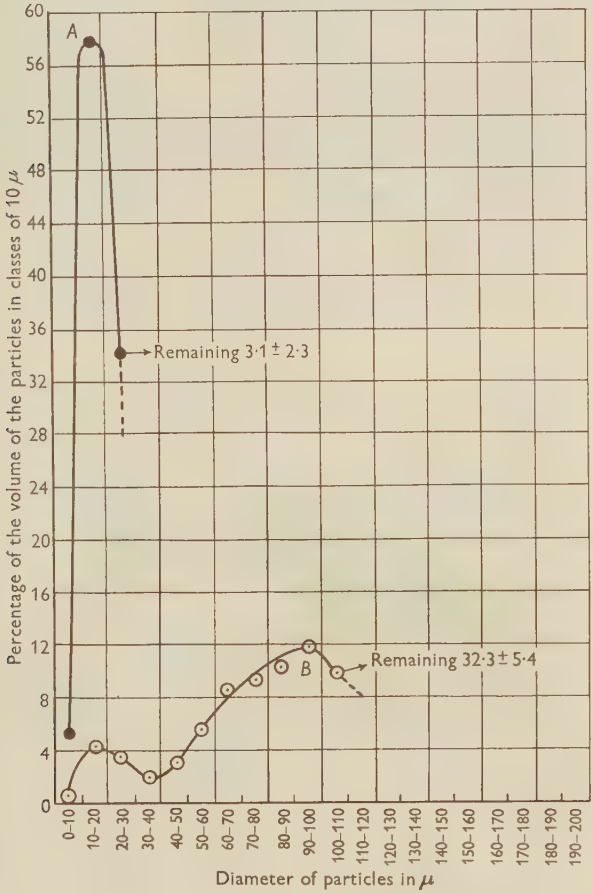
Spraying machine	Liquid sprayed
A, Helicopter	Light mineral oil
B, Strawsonizer	Light mineral oil

As has already been stated, the frequency curves do not completely characterize the mists, because drops smaller than about 5 μ may remain suspended in the air and are not deposited quantitatively on the glass plates.

Whenever the number of particles per size class dropped to below 30, the frequency curve was broken off. In that case the total number of the remaining particles was stated in the graph at the end of each curve.

The *volume* distribution curves are plotted in Text-fig. 4 for the sprayings carried

out with the helicopter and the Strawsonizer. The data obtained with the laboratory spraying apparatus have been plotted in Text-fig. 5.



Text-fig. 5. Volume distribution of spray droplets produced by laboratory spraying equipment. *A*, normal adjustment for laboratory investigation; *B*, coarsest possible adjustment. Liquid sprayed: light mineral oil.

These curves indicate, *inter alia*, that the, probably, rather large number of very fine particles neglected in our counts (smaller than 5 μ) have no appreciable effect on the percentage dispersion of the volume of oil. They show, moreover, that a rather small number of coarse particles, as formed in the helicopter tests and the laboratory tests with a coarse mist, may represent a very considerable part of the spray volume.

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Oil spots deposited on glass plates coated with Drifilm (magnification 40 times).

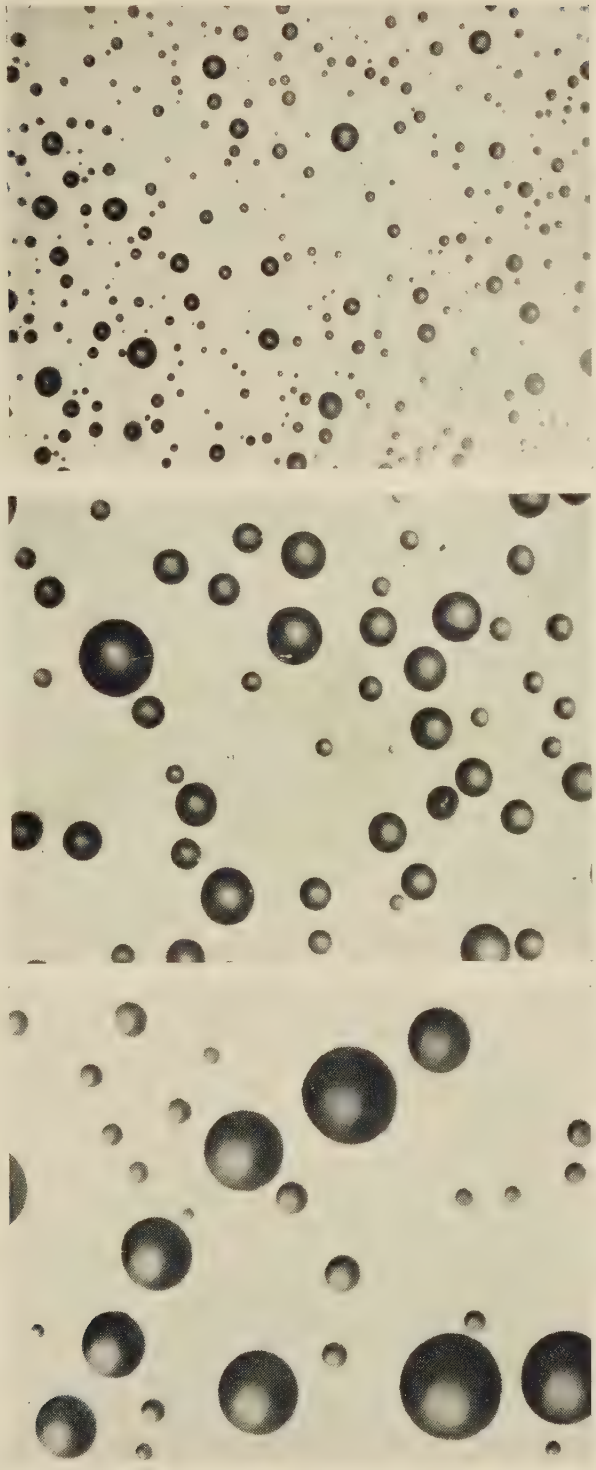

Fig. 1. Mineral oil spray from helicopter fitted with a spraying boom and Lyunet atomizing nozzles.

Fig. 2. Mineral oil spray from Strawsonizer.

Fig. 3. Mineral oil spray from laboratory apparatus when normally adjusted.

STATISTICAL ASPECTS OF THE INDEPENDENT JOINT ACTION OF POISONS, PARTICULARLY INSECTICIDES

II. EXAMINATION OF DATA FOR AGREEMENT WITH THE HYPOTHESIS

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(With 2 Text-figures)

Methods are described for discovering whether a mixture of two poisons is as toxic as predicted on the hypothesis of independent joint action. These include χ^2 tests and a procedure for finding the maximum likelihood estimate of the coefficient of correlation in resistance to the poisons. The methods are illustrated using data from insecticidal tests.

In the insecticidal tests, flour beetles, *Tribolium castaneum* Herbst, were sprayed with, or exposed to films of, different insecticides in solution in Shell oil P 31. The insecticides were pyrethrins, D.D.T., and B.H.C., singly and in pairs. The statistical analysis of the results showed that pyrethrins and D.D.T. could have acted independently both as films and as direct sprays; D.D.T. and B.H.C. could have acted independently as films but not as direct sprays; and B.H.C. and pyrethrins could not have acted independently either as films or as direct sprays.

These findings are discussed, and it is concluded that independent action should be regarded as a special case of a more general type of joint action for which the term *dissimilar* is proposed. A general method of approach is suggested for the conception and development of hypotheses of the joint action of poisons.

Introduction

In a previous paper (Plackett & Hewlett, 1948), we described how to predict the toxicity of a mixture of two poisons from those of its components, assuming that the components act independently. In this paper we are mainly concerned with methods for discovering whether or not a mixture of poisons is as toxic as predicted.

By means of statistical tests it is possible to say whether observed mortalities due to a mixture agree with those predicted, within the limits of sampling error. If they do agree, either the action of the poisons is physiologically independent, or it is not independent but happens to give approximately the same mortalities as would independence. If, on the other hand, the mixture mortalities observed do not agree with those predicted, either the poisons do not act independently, or the mixture mortalities are heterogeneous. Heterogeneity apart, statistical analysis can, in fact, only indicate that poisons may be acting independently, or that they do not, but

not that they do. It provides contradictory or permissive evidence, but not indicative. Nevertheless, the more extensive a set of data compatible with the hypothesis of independent action, the less likely is it to be compatible with another hypothesis.

Heterogeneity in the mixture mortalities renders them unsuitable for testing the hypothesis of independent action, and each set of mixture mortalities should be examined for heterogeneity before analysing it statistically. Heterogeneity will usually be evident from the scatter of points when the mixture mortalities are plotted against dose. It can be expected to be absent if the experimental technique is sound, and homogeneous results are normally obtained for the separate poisons.

It is envisaged that dosage-mortality data are obtained for each poison separately, and for one or more mixtures of poisons. If concentration is varied, the mixture may contain the poisons in any proportion, and the results remain suitable for statistical analysis. If deposit is varied, however, the mixture must contain the same amounts of the poisons as the preparations containing the poisons separately, unless it can be shown that a given amount of active constituent produces the same response irrespective of the concentration at which it is applied: for example, if poison A is used in 2 % solution and poison B in 1 % solution, the mixture must contain 2 % of A plus 1 % of B.

The mathematical basis for the statistical procedures described in this paper is not given, but it is hoped to publish this elsewhere.

The toxicities of the components of a mixture

It is assumed that toxicities of the separate poisons can be expressed by the equations†

$$y_1 = \gamma_1 + \beta_1(\log z - \overline{\log z_1}), \tag{14}$$

$$y_2 = \gamma_2 + \beta_2(\log z - \overline{\log x_2}), \tag{15}$$

where, for example, $\overline{\log z_1}$ is the weighted mean of the log-doses for the first poison. In these equations, y_1 and y_2 are normal equivalent deviations (N.E.D.'s), but probit tables can be employed in calculating the best estimates $\bar{y}_1$, $\bar{y}_2$, b_1, b_2 of γ_1, γ_2, β_1, β_2 respectively. As probits are merely N.E.D.'s + 5, the rules for computing probit regression lines are followed, subtracting 5 either throughout or at the end, according to choice. Finney (1947) has described comprehensively the calculation of probit-log-dose regression equations.

If z is concentration, then from equations (14) and (15):

$$x_1 = \gamma_1 + \beta_1[\log(\pi_1 z) - \overline{\log z_1}] = \xi_1 + \beta_1(\log z - \overline{\log z_1}), \tag{14a}$$

$$x_2 = \gamma_2 + \beta_2[\log(\pi_2 z) - \overline{\log z_2}] = \xi_2 + \beta_2(\log z - \overline{\log z_2}). \tag{15a}$$

(This unusual notation, x_1 and x_2 as dependent variables, originates in equation (9) of the previous paper, where they are independent variables.) Thus, if $k_1 = \bar{y}_1 + b_1 \log \pi_1$ is the estimate of ξ_1, and k_2 that of ξ_2,

$$V(k_1) = V(\bar{y}_1) + (\log \pi_1)^2 V(b_1),$$

† For equations (1)–(13), see the previous paper (Plackett & Hewlett, 1948).

and $V(x_1)$ is estimated by

$$V(x_1) = V(k_1) + (\log z - \overline{\log z_1})^2 V(b_1).$$

$V(k_2)$ and $V(x_2)$ are found correspondingly. If z is deposit $x_1 = y_1$ and $x_2 = y_2$, so $V(x_1) = V(y_1)$ and $V(x_2) = V(y_2)$.

Tests of significance

The proportions observed killed by the mixture at different doses are corrected for control mortality, giving quantities p^*: these are compared with the predicted mortalities, p, by means of χ^2 tests. The χ^2's are of two kinds:

(i) Unadjusted χ^2, written χ^2_u. This is given by the formula

$$\chi^2_u = S\left[\frac{n(p^*-p)^2}{q\left(p+\frac{c}{1-c}\right)}\right], \tag{16}$$

where S denotes summation for the different doses, n is the number of insects in the batch from which p^* is derived and c is the control mortality expressed as a proportion. Alternatively,

$$\chi^2_u = S[w(x^*-x)^2], \tag{17}$$

where x^* and x are the N.E.D.'s corresponding to p^* and p respectively, and w is the weight of the observation, i.e. n times the weighting coefficient corresponding to x (adjusted for control mortality as in Finney (1947), Table II). Whichever equation is used, groups in which the expected numbers dead or alive, i.e. np or nq, are less than the conventional 5, should be combined. For this reason equation (16) is generally preferable, and it is particularly so here because when $\rho = +1, 0$, or -1, p is simply expressed in terms of p_1 and p_2.

Bliss (1935, p. 310) states, in effect, that equation (17) with $c = 0$ gives a larger value for χ^2 than equation (16). This is incorrect. The values given by the two equations differ slightly, and sometimes that given by equation (17) is larger and at others smaller.

(ii) Adjusted χ^2, written χ^2_a. The unadjusted χ^2 does not allow for the error to which p is subject, due to the indeterminacy of p_1 and p_2, and is therefore too critical of the fit. Thus, if χ^2_u is calculated, and the corresponding value for P is read from an ordinary χ^2 table and found to be 0·1, the true value of P is greater than 0·1: the observed mortalities agree with those predicted within the limits of sampling error. If, however, the value of P from the ordinary χ^2 table is less than 0·05, the true value of P may exceed 0·05. The adjusted χ^2 attempts to allow for the error in p and so to indicate a value of P nearer the true value.

χ^2_a can only be used when the data for the separate poisons are homogeneous, and is given, in terms of proportions killed, by

$$\chi^2_u = S\left[\frac{n(p^*-p)^2}{q\left(p+\frac{c}{1-c}\right)+nV(p)}\right]. \tag{18}$$

Equation (18) differs from equation (16) in having a correction term, $nV(\bar{p})$, in the denominator. The method of calculating this term depends upon the value of ρ and also upon whether groups must be combined due to low expectations. The calculation of the correction term is now given only for $\rho = +1$, 0, and -1, because its calculation for other values of ρ would be complicated.

Where the expectations are greater than 5,

$$\text{for } \rho = +1, \quad V(\bar{p}) = V(p_1) \text{ or } V(p_2), \quad \text{as } \bar{p} = p_1 \text{ or } p_2;$$

$$\text{for } \rho = 0, \quad V(\bar{p}) = q_2^2 V(p_1) + q_1^2 V(p_2);$$

and

$$\text{for } \rho = -1, \quad V(\bar{p}) = V(p_1) + V(p_2).$$

$V(p_1) = Z_1^2 V(x_1)$ and $V(p_2) = Z_2^2 V(x_2)$, Z_1 and Z_2 being the ordinates of the normal curve corresponding to the N.E.D.'s x_1 and x_2 respectively.

Where the expectations are less than 5 the correction term cannot be obtained so simply. For this reason it often may not be worth while to include in χ_a^2 observations with such low expectations.

When $\rho = +1$, $\bar{p} = p_1$ or p_2. Suppose, in fact, that s expectations derived from the regression line for poison A alone must be combined. Assume for convenience that n is constant for all groups: n usually varies little and a typical value will do. Then replace n by sn, $\bar{p}^*$ and p_1 by their respective averages over s values, and $V(p_1)$ by

$$1/s^2\{(S_1 Z_1)^2 V(k_1) + [S_1 Z_1(\log z - \overline{\log z_1})]^2 V(b_1)\}, \tag{19}$$

the summation S being taken over the constituent subgroups, and z denoting the dose applied in a particular subgroup. Where $\rho = 0$, for groups formed by combination replace n, $\bar{p}^*$, and $\bar{p}$ as for $\rho = +1$, and $V(\bar{p})$ by

$$1/s^2\{(S_1 Z_1 q_2)^2 V(k_1) + [S_1 Z_1 q_2(\log z - \overline{\log z_1})]^2 V(b_1) + (S_2 Z_2 q_1)^2 V(k_2) + [S_2 Z_2 q_1(\log z - \overline{\log z_2})]^2 V(b_2)\}. \tag{20}$$

Where $\rho = -1$, for groups formed by combination again replace n, $\bar{p}^*$, and $\bar{p}$ as for $\rho = +1$, and $V(\bar{p})$ by the sum of equation (19) and a similar term for the second poison. This procedure will tend to exaggerate the correction term, i.e. to underestimate χ_a^2.

Degrees of freedom. The number of degrees of freedom (D.F.) is the same for χ_u^2 and χ_a^2, but needs to be decided with care. Three situations can arise:

(i) A set of mixture mortalities is to be tested for agreement with the hypothesis of independent action after the maximum likelihood estimate for ρ has been computed from the data. The D.F. is then the number of groups (after combination to avoid low expectations) less one, as one parameter has been estimated from the mixture mortalities.

(ii) A set of mixture mortalities is to be tested for independent action with a value of ρ decided without reference to them; that is to say, decided either arbitrarily or from previous sets of data. The D.F. is then the number of groups.

(iii) A set of mixture mortalities is to be tested for independent action with

$\rho = +1$, 0, or -1, whichever a graphical inspection of the mortalities indicates as giving the best fit. The value of ρ selected from amongst $+1$, 0, and -1 is very unlikely to be the maximum likelihood estimate, so that the number of groups less one is too few for the D.F. On the other hand, the number of groups is too many for the D.F., because the value of ρ chosen partly depends on the data. It is to be hoped that acceptance or rejection of the hypothesis will not depend on a difference of one in the D.F., and in any case it should be remembered that the maximum value of P for significance, say 0·05, is arbitrary.

P from χ_a^2 should be taken as lying between the value indicated for the D.F. equal to the number of groups and the value for one less than this number. P from χ_u^2 is smaller than the value corresponding to a D.F. equal to the number of groups.

Procedure of analysis

The general procedure for the analysis of a set of mixture mortalities is now given. The details of the computations are delayed until the results of some insecticidal tests have been presented, so that these details can be illustrated by concrete examples.

(i) The results for the mixture are examined for homogeneity by plotting the observed mixture N.E.D.'s against log-dose, and drawing a smooth curve like a parabola through them. If it seems doubtful whether they are homogeneous, this is roughly tested by calculating a χ^2 in the same way as χ_u^2, with D.F. three less than the number of groups. If this χ^2 is significant, the mixture mortalities are unsuitable for further analysis.

(ii) The second step is to calculate the individual regression lines giving y_1, y_2, x_1, and x_2, together with the errors of their parameters.

(iii) The third step is to discover whether the observed mixture mortalities, p^*, lie in the right range for independent action; that is, between the greater of p_1 and p_2 and the lesser of (p_1+p_2) and 1. The observed mixture mortality for a given dose is taken; the values of x_1 and x_2 at that dose are calculated from the regression lines; p_1 and p_2 are found from x_1 and x_2 respectively; and the value of p^* is compared with the greater of p_1 and p_2 and the lesser of (p_1+p_2) and 1. The process is carried out for each mixture mortality. There are four possibilities:

(*a*) All the values of p^* are in the right range for independent action. If all that is then required is to know whether or not the action can be independent with $\rho = +1$, 0, or -1, χ_u^2 and/or χ_a^2 (equations (16)–(20)) are calculated. If χ_a^2 for $\rho = +1$, 0, or -1 is significant, the action may still be independent, ρ having another value; if χ_u^2 or χ_a^2 is not significant, estimating ρ will almost certainly give estimates of p nearer to p^*. The procedure outlined in (iv) gives an estimate, r, of ρ which will be unbiased if samples are large enough. (This r is different from Bliss's (1939); r is the usual symbol for the estimate of the bivariate normal correlation coefficient.) Having calculated r, the values of p^* and p (obtained using r) are compared by calculating χ_u^2.

(*b*) The values of p^* all lie above or all lie below the range for independent action.

If all lie above, whether they are significantly so can be tested by calculating χ_u^2 and/or χ_a^2 for $\rho = -1$. If χ_a^2 is not significant, the action can be independent with $\rho = -1$, and -1 will be the best estimate of ρ. If χ_a^2 is significant, the action certainly cannot be independent, since χ_a^2 will be too small if anything. Similar tests are carried out for $\rho = +1$ when all values of p^* lie below the range.

(*c*) Some of the values of p^* are within the range and the others outside it. Usually ρ is estimated as in (iv) and χ_u^2 is calculated. If χ_u^2 is not significant, the action can be independent, but otherwise not. However, some of the values for p^* may depart so far from p for $\rho = -1$ or $+1$ that alone they provide a χ^2 significant on all the degrees of freedom available, so that the estimation of ρ is unnecessary.

(*d*) Some of the values of p^* are above the range, and the rest below it. This is an unlikely contingency, but should it occur, proceed as under (*c*).

(iv) Estimation of ρ. Provided that the parameters of the individual regression lines are not subject to large errors, the maximum likelihood estimate for ρ occurs when

$$G = S\left[\frac{\psi n(p^* - p)}{q\left(p + \frac{c}{1-c}\right)}\right] = 0,$$

where

$$\psi = \frac{1}{2\pi\sqrt{(1-r^2)}} \exp\left[-\frac{1}{2(1-r^2)}(x_1^2 - 2x_1x_2r + x_2^2)\right]. \tag{21}$$

The value of r is not computed directly from the data. Instead, G is calculated for a number of values of r over a range including the value for which $G = 0$, and the best estimate of r found by interpolation. The values of r for which G should be calculated can be judged by first sketching the mixture curves for $\rho = -1$ and 0.

The variance of r, provided χ_u^2 is not significant, is estimated by

$$V(r) = \left\{S\left[\frac{n\psi^2}{q\left(p + \frac{c}{1-c}\right)}\right]\right\}^{-1}. \tag{22}$$

If χ_u^2 is significant this estimate must be multiplied by the heterogeneity factor, χ_u^2 divided by the D.F.

(v) A procedure theoretically more satisfactory than that just described would be to estimate $\gamma_1, \gamma_2, \beta_1, \beta_2$, and ρ *simultaneously* from the three sets of observations, two for the separate poisons and one for the mixture. An overall χ^2 for goodness of fit could then be calculated with degrees of freedom 5 less than the total number of doses. The computation involved, however, would be formidable and we have not attempted it. In any event it would be inadvisable unless the data for the separate poisons were homogeneous, and χ_u^2, found after estimating ρ as in (iv), indicated good agreement.

Tests on the joint action of pyrethrins, D.D.T., and benzene hexachloride taken in pairs

Experiments were made to test the joint action of pyrethrins, D.D.T., and γ-benzene hexachloride, taken in pairs, on flour beetles. The data obtained were analysed by the methods just described, which they serve to illustrate. The data were not extensive enough to be entirely conclusive, but the tests were valuable in suggesting how independent action could be modified.

The beetles were *Tribolium castaneum* Herbst, P.I.L. Stock no. 5, and were 3–5 weeks old when used. The insecticides were solutions of pyrethrins, D.D.T., or γ-B.H.C., in Shell oil P31; and solutions containing each pair of the three materials. The solutions including pyrethrins were prepared by diluting a concentrate in P31, containing 2·72 and 2·81 % w/v of pyrethrin I and II respectively. The D.D.T. was the pure *p*, *p*′-compound. The B.H.C. was the pure γ-isomer. The beetles were exposed on films, formed on Whatman no. 544 filter-paper, by the method of Parkin & Green (1943); or were directly sprayed by the method of Hewlett (1947).

Each experiment was a complete comparison in itself, for in each beetles were treated by one method with two active ingredients separately and together. The deposit was varied, and, as it was known that the toxicity of a given amount of these active ingredients varies according to the concentration at which they are applied (see Hewlett, 1948), the mixture solutions contained the same concentrations of the active ingredients as the corresponding solutions containing one active ingredient.

The solutions containing pyrethrins alone were themselves composite, since they contained more than one toxic ingredient. However, Martin (1943) concluded that pyrethrin I was approximately as lethal to *T. castaneum* as pyrethrin II; and he found that the regression line for an oil solution containing much more pyrethrin I than II was of the same slope as that for a solution containing much more II than I. This suggests that in killing *T. castaneum* the two pyrethrins act similarly in the sense of Bliss (1939). More recently it has been discovered that the pyrethrins (*sensu lato*) each consist of a pyrethrin (*sensu stricto*) and a cinerin; but all four substances gave parallel regression lines when tested against flies (see Gersdorff, 1947), suggesting that all four act similarly. In consequence the pyrethrins were regarded as a single active ingredient in interpreting the tests described here. Even if the pyrethrins do not kill *T. castaneum* by similar action, the tests for independent action between pyrethrins on the one hand, and D.D.T. and B.H.C. on the other, are not necessarily invalid. They would only be invalid if one pyrethrin or cinerin acted independently with D.D.T. or B.H.C., while another acted non-independently.

The results are given in Tables 1–6.

TABLE 1. *The toxicity to* Tribolium castaneum *of films formed by pyrethrins, D.D.T., and the two together, in Shell oil P31*

Insecticide	Deposit (mg./10 sq.cm.)	No. of beetles	Percentage mortality after Six days	Nine days
1·6 % w/v pyrethrins	16·70	50	10	28
	18·29	50	22	34
	19·35	49	24·5	51·0
	20·00	50	26	58
	20·96	50	38	52
	22·26	50	56	78
	22·96	50	86	100
	23·69	50	58	74
0·5 % w/v D.D.T.	12·10	50	6	40
	13·97	50	4	32
	16·05	50	22	44
	18·13	50	26	56
	18·75	50	40	64
	20·31	50	42	70
	21·38	50	56	76
	23·61	50	58	74
1·6 % pyrethrins + 0·5 % D.D.T.	8·49	50	8	32
	10·10	50	8	34
	11·87	50	10	32
	13·84	50	14	42
	15·32	50	18	56
	17·30	50	34	70
	20·52	50	52	74
	22·10	50	64	76
Control	—	200	0·0	1·5

Nine days:	S.E. of b	χ^2	D.F.	P (approx.)
Pyrethrins $x_1 = 0{\cdot}076 + 9{\cdot}270\ (\log z - 1{\cdot}302)$	1·540	4·78	5	0·44
D.D.T. $x_2 = 0{\cdot}163 + 4{\cdot}250\ (\log z - 1{\cdot}246)$	0·736	5·65	6	0·46

TABLE 2. *The toxicity to* Tribolium castaneum *of direct sprays of pyrethrins, D.D.T., and the two together, in Shell oil P31*

Insecticide	Deposit (mg./10 sq.cm.)	No. of beetles	Percentage mortality after Six days	Nine days
1·2 % w/v pyrethrins	2·52	48	6·2	12·5
	3·30	48	6·2	12·5
	4·25	50	18	28
	5·33	50	32	38
	7·15	50	40	58
	9·53	50	60	80
	12·28	49	75·5	95·9
	15·58	50	70	90

TABLE 2 (*continued*)

2·0% w/v D.D.T.	2·45	49	16·3	22·5
	3·18	50	16	26
	4·25	50	32	40
	5·48	50	42	54
	7·24	50	50	64
	9·54	50	56	72
	12·36	50	70	86
	15·54	50	74	86
1·2% pyrethrins + 2·0% D.D.T.	2·47	50	28	38
	3·20	49	36·7	46·9
	4·10	50	44	66
	5·34	50	72	90
	7·11	50	84	94
	9·60	50	90	96
	12·45	50	100	100
	15·65	50	100	100
Control	—	200	0·0	2·0

	S.E. of b	χ^2	D.F.	P (approx.)
Six days:				
Pyrethrins $x_1 = -0{\cdot}237 + 3{\cdot}008$ (log $z - 0{\cdot}849$)	0·300	4·89	5	0·43
D.D.T. $x_2 = -0{\cdot}121 + 2{\cdot}150$ (log $z - 0{\cdot}817$)	0·264	1·59	6	0·96
Nine days:				
Pyrethrins $x_1 = 0{\cdot}054 + 3{\cdot}956$ (log $z - 0{\cdot}804$)	0·353	1·65	4	0·79
D.D.T. $x_2 = 0{\cdot}136 + 2{\cdot}553$ (log $z - 0{\cdot}786$)	0·282	1·26	6	0·98

TABLE 3. *The toxicity to* Tribolium castaneum *of films formed by D.D.T., γ-benzene hexachloride, and the two together, in Shell oil P31*

Insecticide	Deposit (mg./10 sq.cm.)	No. of beetles	Percentage mortality after Six days	Nine days
0·5% w/v D.D.T.	11·79	50	8	26
	13·76	50	18	50
	15·58	50	24	34
	17·84	50	56	68
	19·74	49	65·3	75·5
	21·90	50	78	84
0·1% w/v γ-B.H.C.	12·08	50	30	40
	14·49	49	49·0	57·1
	16·31	50	52	66
	18·13	50	48	60
	20·44	50	58	66
	22·26	49	59·2	67·3
0·5% D.D.T. + 0·1% γ-B.H.C.	12·05	50	54	64
	13·61	48	62·5	68·7
	16·08	50	72	86
	18·00	50	78	84
	19·74	50	80	86
	22·23	50	94	96
Control	—	200	2·0	3·0

	S.E. of b	χ^2	D.F.	P (approx.)
Six days:				
D.D.T. $x_1 = -0{\cdot}127 + 9{\cdot}040$ (log $z - 1{\cdot}239$)	1·108	2·73	3	0·45
B.H.C. $x_2 = -0{\cdot}040 + 2{\cdot}529$ (log $z - 1{\cdot}230$)	0·847	2·21	4	0·69
Nine days:				
D.D.T. $x_1 = 0{\cdot}133 + 6{\cdot}078$ (log $z - 1{\cdot}214$)	0·910	9·06	4	0·06
B.H.C. $x_2 = 0{\cdot}205 + 2{\cdot}424$ (log $z - 1{\cdot}227$)	0·851	2·58	4	0·62

TABLE 4. *The toxicity to* Tribolium castaneum *of direct sprays of D.D.T., γ-benzene hexachloride, and the two together, in Shell oil P31*

Insecticide	Deposit (mg./10 sq.cm.)	No. of beetles	Percentage mortality after Six days	Nine days
2·0% w/v D.D.T.	2·00	50	6	12
	2·64	49	10·2	18·4
	3·48	47	40·4	46·8
	4·59	50	38	44
	6·06	49	49·0	55·1
	8·00	50	70	80
1·5% w/v γ-B.H.C.	2·00	50	4	6
	2·64	49	28·6	34·7
	3·48	50	40	50
	4·59	50	54	60
	6·06	50	82	86
	8·00	50	80	84
2·0% D.D.T.+1·5% γ-B.H.C.	2·00	50	56	58
	2·64	50	74	76
	3·48	50	92	96
	4·59	50	96	100
	6·06	50	96	98
	8·00	50	100	100
Control	—	200	1·0	2·0

	S.E. of b	χ^2	D.F.	P (approx.)
Six days:				
D.D.T. $x_1 = 0{\cdot}335 + 3{\cdot}391\ (\log z - 0{\cdot}660)$	0·454	2·56	3	0·46
B.H.C. $x_2 = 0{\cdot}033 + 3{\cdot}973\ (\log z - 0{\cdot}616)$	0·447	7·74	4	0·10
Nine days:				
D.D.T. $x_1 = 0{\cdot}180 + 3{\cdot}231\ (\log z - 0{\cdot}638)$	0·433	3·17	4	0·52
B.H.C. $x_2 = 0{\cdot}075 + 4{\cdot}000\ (\log z - 0{\cdot}601)$	0·450	8·55	4	0·07

TABLE 5. *The toxicity to* Tribolium castaneum *of films formed by γ-benzene hexachloride, pyrethrins, and the two together, in Shell oil P31*

Insecticide	Deposit (mg./10 sq.cm.)	No. of beetles	Percentage mortality after Six days	Nine days
0·1% w/v γ-B.H.C.	16·21	50	46	60
	17·30	50	56	70
	18·54	49	40·8	51·0
	20·49	48	60·4	72·9
	22·29	50	56	66
	24·44	50	62	68
1·6% w/v pyrethrins	15·84	50	4	8
	17·71	50	8	26
	18·52	51	11·8	41·2
	20·41	50	40	54
	22·00	50	56	72
	23·82	50	94	98

TABLE 5 (*continued*)

0·1 % γ-B.H.C.+	16·18	50	66	80
1·6 % pyrethrins	17·09	50	78	90
	18·29	50	82	90
	20·34	49	79·6	85·7
	22·13	50	90	100
	23·32	50	98	100
Control	—	200	0·0	1·5

		S.E. of b	χ^2	D.F.	P (approx.)
Six days:					
B.H.C.	$x_1 = 0{\cdot}083 + 2{\cdot}037$ (log $z - 1{\cdot}293$)	1·179	3·63	4	0·45
Pyrethrins	$x_2 = 0{\cdot}228 + 18{\cdot}588$ (log $z - 1{\cdot}310$)	1·886	4·64	3	0·20
Nine days:					
B.H.C.	$x_1 = 0{\cdot}361 + 1{\cdot}153$ (log $z - 1{\cdot}293$)	1·216	5·81	4	0·21
Pyrethrins	$x_2 = 0{\cdot}037 + 16{\cdot}122$ (log $z - 1{\cdot}295$)	1·718	2·05	2	0·36

TABLE 6. *The toxicity to* Tribolium castaneum *of direct sprays of γ-benzene hexachloride, pyrethrins, and the two together, in Shell oil P31*

Insecticide	Deposit (mg./10 sq.cm.)	No. of beetles	Percentage mortality after: Six days	Nine days
1·5 % w/v γ-B.H.C.	2·00	50	22	24
	2·64	50	30	36
	3·48	48	56·2	60·4
	4·59	50	62	72
	6·06	50	70	80
	8·00	50	74	78
1·2 % w/v pyrethrins	2·00	49	2·0	6·1
	2·64	50	6	10
	3·48	50	24	28
	4·59	48	24·5	31·3
	6·06	50	38	48
	8·00	50	46	54
1·5 % γ-B.H.C.+	1·98	50	62	70
1·2 % pyrethrins	2·62	50	78	80
	3·45	50	94	96
	4·55	50	94	98
	6·00	50	98	100
	7·93	49	100	100
Control	—	197	0·5	1·0

		S.E. of b	χ^2	D.F.	P (approx.)
Six days:					
B.H.C.	$x_1 = 0{\cdot}048 + 2{\cdot}481$ (log $z - 0{\cdot}599$)	0·385	3·63	4	0·46
Pyrethrins	$x_2 = -0{\cdot}646 + 2{\cdot}869$ (log $z - 0{\cdot}678$)	0·471	4·49	3	0·21
Nine days:					
B.H.C.	$x_1 = 0{\cdot}184 + 2{\cdot}748$ (log $z - 0{\cdot}586$)	0·398	5·32	4	0·26
Pyrethrins	$x_2 = -0{\cdot}482 + 2{\cdot}812$ (log $z - 0{\cdot}662$)	0·445	2·51	3	0·48

Analysis of results

Computation of regression equations

N.E.D.-log-dose regression equations were computed from the sets of data for the separate poisons. These equations are given under Tables 1–6, together with χ^2's for goodness of fit, etc. The equations were obtained from two, or in a few cases three, cycles of computations. The N.E.D.'s calculated from each can be expected to be within, usually well within, 0·01 of the maximum likelihood N.E.D.'s, over the range of doses covered in the corresponding data.

In Table 1 the mortalities for 22·96 mg./10 sq.cm. of 1·6 % pyrethrins were unduly high, and were ignored in computing the corresponding regression equations.

Tests for independent action

The sets of data were tested for agreement with the hypothesis of independent action. Tables 1, 3 and 5 show that in the film tests the regression coefficients for the three poisons differed significantly. The coefficients in Table 2 for direct sprays of pyrethrins and D.D.T. do not differ significantly, but other, unpublished data show that the difference between the coefficients is real. Thus pyrethrins, D.D.T. and B.H.C. as films, and pyrethrins and D.D.T. as direct sprays, could not act similarly as defined by Bliss (1939). D.D.T. and B.H.C., and B.H.C. and pyrethrins, as direct sprays might have acted similarly since the regression coefficients do not differ significantly. These data, however, are not suitable for testing for agreement with the hypothesis of similar action: they relate mortality to deposit, but should relate it to concentration in order to test for similar action.

The results for the different pairs of insecticides are now considered. None of the sets of mixture mortalities was heterogeneous. Where a χ_a^2 is given, the corresponding χ_u^2 is also given for comparison. The values of P were determined with the help of the chart prepared by Bliss (1944).

Pyrethrins and D.D.T., films (see Table 1)

The design of this experiment was defective. The three lowest doses of the mixture were below the range of doses used for the separate insecticides, and so were ignored in analysing the results. For this reason only the 9-day mortalities were analysed. In succeeding experiments the doses of the mixtures were kept in the same range as those for the corresponding separate insecticides.

All the 9-day mixture mortalities were in the right range for independent action. It was found that $r = +0{\cdot}66 \pm 0{\cdot}19$: $\chi_u^2 = 4{\cdot}43$. This value of χ^2 on 4 D.F. gives $P = 0{\cdot}35$, but as χ_u^2 indicates a higher value for P than that found from the tables, P is really greater than 0·35. For $\rho = +1$, $\chi_a^2 = 5{\cdot}96$ on 4 or 5 D.F., whence $0{\cdot}20 < P < 0{\cdot}30$: $\chi_u^2 = 6{\cdot}92$.

Pyrethrins and D.D.T., direct sprays (*see Table 2 and Fig. 1*)

Six days. All the mixture mortalities were in the right range for independent action except those for 2·47 and 3·20 mg./10 sq.cm., which were a little above. It was found that $r = -0{\cdot}74 \pm 0{\cdot}09$: $\chi_u^2 = 3{\cdot}98$ on 4 D.F., whence $P > 0{\cdot}41$.

Nine days. All the mixture mortalities were in the right range except those for 2·47 and 5·34 mg./10 sq.cm., which were slightly above. It was found that $r = -0{\cdot}59 \pm 0{\cdot}13$: $\chi_u^2 = 5{\cdot}67$ on 3 D.F., whence $P > 0{\cdot}12$.

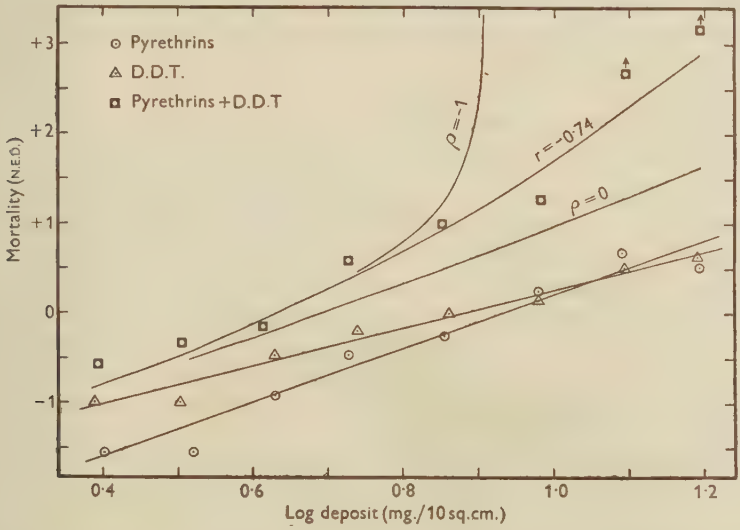

Fig. 1. The toxicity to *T. castaneum* of direct sprays of 1·2% pyrethrins, 2·0% D.D.T., and the two together: 6-day results. ↑ indicates a complete mortality. The mixture mortalities are well fitted by the hypothesis of independent action, with r, the maximum likelihood estimate of ρ, equal to $-0{\cdot}74$. The mixture curves for $\rho = 0$ and $\rho = -1$ are also shown.

D.D.T. and B.H.C., films (*see Table 3*)

Six days. All the mixture mortalities were in the right range. It was found that $r = -0{\cdot}16 \pm 0{\cdot}18$: $\chi_u^2 = 9{\cdot}22$ on 4 D.F., whence $P > 0{\cdot}058$. For $\rho = 0$, $\chi_a^2 = 7{\cdot}02$ on 4 or 5 D.F., whence $0{\cdot}14 < P < 0{\cdot}22$: $\chi_u^2 = 9{\cdot}84$. It is therefore concluded that for $r = -0{\cdot}16$, $P > 0{\cdot}14$.

Nine days. All the mixture mortalities were again in the right range. It was found that $r = 0{\cdot}00 \pm 0{\cdot}16$: $\chi_u^2 = 2{\cdot}33$ on 4 D.F., whence $P > 0{\cdot}68$. As the maximum likelihood estimate for ρ happens to be zero, χ_a^2 for $r = 0$ could be calculated, but this was not worth while since the value of χ_u^2 is small enough to show that the action could have been independent.

D.D.T. and B.H.C., direct sprays (*see Table* 4)

Six days. The mixture mortalities for the four lowest doses are above the range for independent action, those for the three lowest well above, as a glance at the table shows: the two remaining mortalities are within the range. From the three lowest doses only, for $\rho = -1$, $\chi_a^2 = 76{\cdot}6$, ample on 6 D.F., the maximum possible, to reject the hypothesis of independent action with P very much less than 0·001: $\chi_u^2 = 110{\cdot}4$. Taking a higher value of ρ will merely increase the contribution to χ_a^2 given by the mortalities for the three lowest doses.

Nine days. The mixture mortalities for the three lowest doses are very much above the range, as are the 6-day. From the three lowest doses only, for $\rho = -1$, $\chi_a^2 = 38{\cdot}8$, whence $P < 0{\cdot}001$: $\chi_u^2 = 59{\cdot}2$.

B.H.C. and pyrethrins, films (*see Table* 5)

Six days. The mixture mortalities for the three lowest doses are well above the range. From these three doses, for $\rho = -1$, $\chi_a^2 = 18{\cdot}3$. This value of χ^2 on 6 D.F. corresponds to $P = 0{\cdot}0056$. The contributions to χ_a^2 that the other mixture mortalities would make are not included in the value of 18·3, so probably $P < 0{\cdot}001$: $\chi_u^2 = 25{\cdot}9$.

Nine days. The hypothesis of independent action cannot be ruled out so simply here. The mixture mortalities for the two lowest doses are above the range, but for $\rho = -1$ these two give $\chi_u^2 = 6{\cdot}41$, which on 6 D.F. is not significant. Here $r = -0{\cdot}62$: $\chi_u^2 = 9{\cdot}24$ on 2 D.F., corresponding to $P = 0{\cdot}010$ and rejecting the hypothesis of independent action.

B.H.C. and pyrethrins, direct sprays (*see Table* 6 *and Fig.* 2)

Six days. The mixture mortalities for the four lowest doses are above the right range, especially those for the three lowest. From these four doses, for $\rho = -1$, $\chi_a^2 = 56{\cdot}1$, ample on 6 D.F. to rule out the hypothesis of independent action with $P < 0{\cdot}001$: $\chi_u^2 = 80{\cdot}5$.

Nine days. The mixture mortalities for the three lowest doses are well above the range, and from these, for $\rho = -1$, $\chi_a^2 = 38{\cdot}4$, whence $P < 0{\cdot}001$: $\chi_u^2 = 58{\cdot}1$.

Over the whole set of tests the 6-day and 9-day results yielded very similar conclusions. The values for χ_a^2 were not very much lower than those for χ_u^2, because the variances of p_1 and p_2 were small. Had the data for the separate poisons been based on smaller numbers of insects the variances of p_1 and p_2 would have been greater, and the differences between χ_a^2 and χ_u^2 greater also. Where the mixture mortalities were incompatible with the hypothesis of independent action ($P < 0{\cdot}05$), this was because the hypothesis underestimated the mortalities at lower doses. Even where the insecticides could have acted independently, it was noticeable that at lower doses the predicted mixture mortalities, for the maximum likelihood estimate of ρ, were below those observed. The reason for this is being investigated.

Under the particular conditions of test, B.H.C. and pyrethrins were the most

effective combination. At lower doses the hypothesis of independent action massively underestimated the mortalities produced by the mixtures, both in the film and direct spray tests. The pyrethrins appeared to knock the beetles down as rapidly when B.H.C. was present as when it was absent.

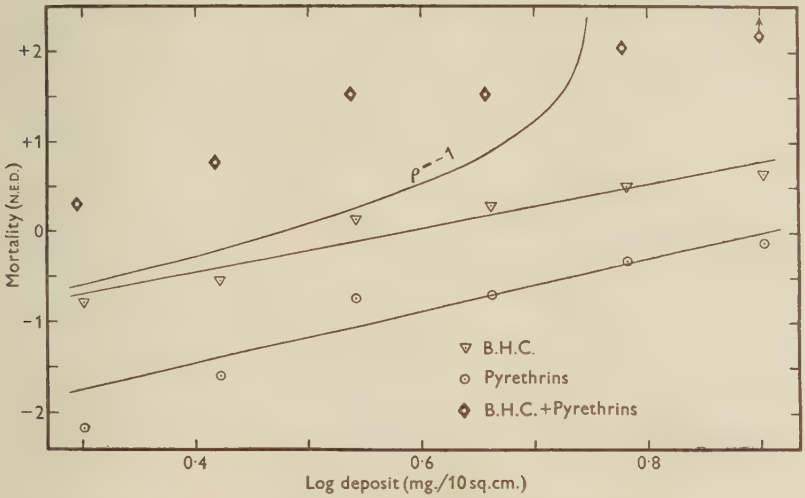

Fig. 2. The toxicity to *T. castaneum* of direct sprays of 1·5% γ-B.H.C., 1·2% pyrethrins, and the two together: 6-day results. The hypothesis of independent action grossly underestimates the mixture mortalities for lower doses: putting $\rho = -1$, the curve for which is shown, gives the highest possible toxicity of the mixture predictable by this hypothesis.

Table 7 summarizes the conclusions from the analysis of the data in Tables 1–6.

TABLE 7. *The probabilities P from testing the hypotheses that pyrethrins, D.D.T., and B.H.C., taken in pairs, acted independently, and, where* $P > 0{\cdot}05$, *the value of r and its standard error*

Insecticide pair	Observation time (days)	Film	Direct spray
Pyrethrins and D.D.T.	6	—	$>0{\cdot}41$ ($r = -0{\cdot}74 \pm 0{\cdot}09$)
	9	$>0{\cdot}35$ ($r = +0{\cdot}66 \pm 0{\cdot}19$)	$>0{\cdot}12$ ($r = -0{\cdot}59 \pm 0{\cdot}13$)
D.D.T. and B.H.C.	6	$>0{\cdot}14$ ($r = -0{\cdot}16 \pm 0{\cdot}18$)	$<0{\cdot}001$
	9	$>0{\cdot}68$ ($r = 0{\cdot}00 \pm 0{\cdot}16$)	$<0{\cdot}001$
B.H.C. and pyrethrins	6	$<0{\cdot}0056$	$<0{\cdot}001$
	9	$>0{\cdot}010$	$<0{\cdot}001$

COMPUTATIONAL DETAILS, ILLUSTRATED BY DATA SELECTED FROM TABLES 1–6

Calculation of χ_u^2 and χ_a^2

The variances of the N.E.D.'s x_1 and x_2 are calculated in the same way as those of probits (see Finney, 1947). $V(p_1)$ is normally given (see p. 530) by $Z_1^2 V(x_1)$: this is readily computed by dividing $V(x_1)$ by $(1/Z_1)^2$, $1/Z_1$ being taken from the tables of Finney & Stevens (1948). $V(p_2)$ is computed similarly. Finney & Stevens tabulate $1/Z$ correct to four decimal places for probits at intervals of 0·01. Linear interpolation in these table suffices, and interpolated values should be found to not more than four decimal places.

As an example of the layout of the computations, the calculation of χ_u^2 and χ_a^2 for $\rho = 0$ is now shown, using the 6-day data for D.D.T. and B.H.C. films from Table 3. Of the three different χ_a^2's, that for $\rho = 0$ is the most complicated to compute. The expected proportion killed by the highest mixture dose was 0·9210; the expected number surviving in a batch of 50 was thus 3·95, so the mortalities for the two highest doses were combined and treated differently from those for the four lowest. The first stage is to complete two sets of columns, one for D.D.T. and one for B.H.C. (Table 8). The left-hand column of each set consists of the mixture doses, and the columns to the right are derived accordingly.

TABLE 8

D.D.T.

Log z	x_1	$V(x_1)$	$1/Z_1^2$	$V(p_1)$	$q_2^2 V(p_1)$
1·0810	−1·5556	0·0378982	70·65808	0·00053636	0·00023484
1·1339	−1·0773	0·0208240	20·05553	0·00103832	0·00038839
1·2063	−0·4228	0·0085848	7·51334	0·00114261	0·00033319
1·2553	+0·0201	0·0075989	6·28559	0·00120894	0·00029110
1·2953	+0·3817	—	—	—	—
1·3470	+0·8491	—	—	—	—

B.H.C.

	x_2	$V(x_2)$	$1/Z_2^2$	$V(p_2)$	$q_1^2 V(p_2)$
1·0810	−0·4172	0·0215968	7·47808	0·00288801	0·00255239
1·1339	−0·2385	0·0122743	6·65116	0·00184544	0·00136267
1·2063	−0·1005	0·0060221	6·34702	0·00094881	0·00041807
1·2553	+0·0234	0·0060573	6·28674	0·00096350	0·00023323
1·2953	+0·1245	—	—	—	—
1·3470	+0·2552	—	—	—	—

The next stage is to derive Table 9 for the calculation of the χ^2's. D_u and D_a are the denominators of χ_u^2 and χ_a^2 respectively (see equations (16) and (18)), i.e. $D_u = q[p + c/(1-c)]$ and $D_a = D_u + nV(p)$. Here the control mortality $c = 0{\cdot}02$, so that $c/(1-c) = 0{\cdot}0204$.

TABLE 9

Log z	n	p^*	p	D_u	$nV(p)$	D_a	χ_u^2	χ_a^2
1·0810	50	0·5306	0·3779	0·24778	0·13936	0·38714	4·7052	3·0115
1·1339	48	0·6173	0·4745	0·26007	0·08405	0·34412	3·7636	2·8444
1·2063	50	0·7143	0·6415	0·23729	0·03756	0·27485	1·1167	0·9641
1·2553	50	0·7755	0·7586	0·18805	0·02622	0·21427	0·0759	0·0666
1·2953 1·3470	100	0·8673	0·8813	0·10703	0·04040	0·14743	0·1831	0·1329
							9·8445	7·0195

For the two highest doses

$$p^* = \tfrac{1}{2}(0{\cdot}7959 + 0{\cdot}9388) = 0{\cdot}8673,$$

$$p = \tfrac{1}{2}(0{\cdot}8417 + 0{\cdot}9210) = 0{\cdot}8813.$$

For the four lowest doses, $nV(p)$ was found simply by adding $q_2^2 V(p_1)$ to $q_1^2 V(p_2)$ and multiplying the sum by n. However, for the two highest doses $nV(p)$ was found by means of equation (20), entailing the following calculations:

log z	$Z_1 q_2$	$Z_1 q_2 (\log z - \overline{\log z_1})$	$Z_2 q_1$	$Z_2 q_1 (\log z - \overline{\log z_2})$
1·2953	0·16710	0·00940773	0·13907	0·00903955
1·3470	0·11108	0·01199664	0·07642	0·00891821
Totals	0·27818	0·02140437	0·21549	0·01795776
Squared totals	0·077384	0·000458	0·04643	0·000322

As z is deposit, $V(k_1) = V(\bar{y}_1)$ and $V(k_2) = V(\bar{y}_2)$, so the squared totals are multiplied by $V(\bar{y}_1)$, $V(b_1)$, $V(\bar{y}_2)$, and $V(b_2)$ respectively, the four products summed, and finally the sum multiplied by n/s^2 to give $nV(p)$. Thus

$$\begin{aligned} nV(p) &= \tfrac{100}{4}(0{\cdot}077384 \times 0{\cdot}007273 + 0{\cdot}000458 \times 1{\cdot}226774 \\ &\quad + 0{\cdot}046436 \times 0{\cdot}005609 + 0{\cdot}000322 \times 0{\cdot}717247) \\ &= 0{\cdot}04040. \end{aligned}$$

Calculation of r

As an example, take the 9-day results of Table 2 for pyrethrins, D.D.T., and pyrethrins with D.D.T., when the insecticides are applied as direct sprays. The N.E.D. regression lines for the individual poisons are:

$$x_1 = -3{\cdot}1265 + 3{\cdot}9556 \log z, \quad x_2 = -1{\cdot}8704 + 2{\cdot}5525 \log z,$$

where x_1 refers to pyrethrins and x_2 to D.D.T. The mixture log-doses range from 2·47 to 15·65, and it is convenient to draw the lines in this interval on probability paper and plot the points corresponding to the mixture. The curve corresponding to $\rho = 0$ is quickly calculated and inspection then suggests a moderate negative value of r as the solution of the maximum likelihood equation; it is decided to try $r = -0{\cdot}60$. The initial steps of the computation are concerned with producing Table 10, which is required for all trial values of r.

TABLE 10

(1) z	(2) $\log z$	(3) x_1	(4) x_2	(5) $x_1^2+x_2^2$	(6) x_1x_2	(7) p_1	(8) p_2	(9) q^*	(10) n
2·47	0·3927	−1·5731	−0·8680	3·2281	1·3655	0·0579	0·1927	0·6327	50
3·20	0·5051	−1·1285	−0·5811	1·6112	0·6558	0·1296	0·2806	0·5306	49
4·10	0·6128	−0·7025	−0·3062	0·5873	0·2151	0·2412	0·3797	0·3469	50
5·34	0·7275	−0·2488	−0·0135	0·0621	0·0034	0·4018	0·4946	0·1020	50
7·11	0·8519	+0·2433	+0·3041	0·1517	0·0740	0·5961	0·6195	0·0612	50
9·60	0·9823	0·7591	0·6369	0·9819	0·4835	0·7761	0·7379	0·0408	50
12·45	1·0952	1·2057	0·9251	2·3095	1·1154	0·8860	0·8225	0	50
15·65	1·1945	1·5985	1·1786	3·9443	1·8840	0·9450	0·8807	0	50

In column (1) the mixture doses are entered, and columns (2)–(8) are derived from column (1). Column (9) consists of the observed proportions surviving the mixture, corrected for control mortality, i.e. divided by the proportion surviving in the controls, here 0·98.

Values of q, given by column (9), must be found for values of ρ other than $+1$, 0, or -1. Tables VIII and IX of Pearson (1931) give values of q for different values of r, x_1 and x_2 (Pearson's r, h and k respectively), but only where x_1 and x_2 are both positive. These tables, however, can be adapted for finding q when x_1 and/or x_2 are negative, and are used according to the following scheme:

x_1	x_2	q
+	+	$T_{+\rho}$
−	−	$1-(p_1+p_2)+T_{+\rho}$
+	−	$q_1-T_{-\rho}$
−	+	$q_2-T_{-\rho}$

$T_{+\rho}$ is the value in the body of the tables for $+\rho$ and the numerical values of x_1 and x_2 (i.e. ignoring sign), and $T_{-\rho}$ that for $-\rho$. For example, if $x_1=+1{\cdot}0$, $x_2=-0{\cdot}5$ and $\rho=-0{\cdot}6$, $T_{-\rho}$ is found from the sub-table for $\rho=+0{\cdot}6$ to be 0·109022, so q is 0·049633. Extensions to Pearson's table have been computed by the Statistical Laboratory of the University of California at Berkeley, but not published. Taken together, the two tables provide values of q for x_1, $x_2=0$, (0·1), 4; $\pm\rho=0(0{\cdot}05)$ 0·95(0·01) 0·99.

The calculation of q, however, is in general a laborious task. Take, for instance, $r=-0{\cdot}60$, $x_1=-1{\cdot}5731$, $x_2=-0{\cdot}8680$: enter Pearson's table of $r=-0{\cdot}60$ and taking k successively equal to 0·8, 0·9, 1·0 and 1·1, find the interpolates corresponding to $h=1{\cdot}5731$, using Newton's method. Thus, multiplying the entries by 10^5, we have:

	$h=1{\cdot}5$		$h=1{\cdot}6$		$h=1{\cdot}7$
$k=0{\cdot}8$	58		40		27
		−18		−13	
			+5		

The proportional part is

$$\theta=0{\cdot}731;\quad \frac{\theta(\theta-1)}{2}=-0{\cdot}098;\quad \frac{\theta(\theta-1)(\theta-2)}{2.3}=0{\cdot}042;$$

whence the interpolate is $58-18(0{\cdot}731)-5(0{\cdot}098)=44$, rounding off.

Proceeding by this method leads to the following table:

k			
0·8	44		
0·9	31	−13	
1·0	22	−9	+4
1·1	16	−6	+3

Interpolation with respect to k completes the process. Here

$$\theta = 0{\cdot}680; \quad \frac{\theta(\theta-1)}{2} = -0{\cdot}109; \quad \frac{\theta(\theta-1)(\theta-2)}{2.3} = 0{\cdot}048,$$

giving $44 - 13(0{\cdot}680) - 4(0{\cdot}109) - 0{\cdot}048 = 34{\cdot}7$. Rounding off to four decimal places, the final interpolate is 0·0003, so $q = 1{\cdot}0003 - 0{\cdot}0579 - 0{\cdot}1927 = 0{\cdot}7497$.

At the head of the next stage of computation (Table 11) are entered the values of $-2r = +1{\cdot}2$, $1/2(1-r^2) = 0{\cdot}78125$, and $1/2\pi\sqrt{(1-r^2)} = 0{\cdot}19894$, the last two being obtained from Table 13. Column (11) consists of the values of q obtained as just described; column (12), $q[p + c/(1-c)]$, is then quickly found. The quantity ϕ in column (13) is $(x_1^2 + x_2^2) - 2r(x_1 x_2)$, being therefore calculated from columns (5) and (6). Column (14) consists of $\psi = \exp(-\phi)/2\pi\sqrt{(1-r^2)}$. To find $\exp(-\phi)$ tables of the descending exponential are convenient but not essential; in their absence a table of natural logarithms will suffice since all that is required is the number whose natural logarithm is $-\phi$: see, for instance, Table XXVI of Fisher & Yates (1949). Thus suppose $\phi = 3{\cdot}8021$, as for the first pair of doses. Add the logarithm of the least power of 10 necessary to convert $-\phi$ from negative to positive: here $\log(100) - \phi = 0{\cdot}8031$, the logarithm of 2·232, giving $\exp(-\phi) = 0{\cdot}02232$ on dividing by the relevant power of 10. It is useful to remember that $\exp(-\phi) = 1 - \phi$ correct to four decimal places when ϕ is less than 0·01 and correct to five when less than 0·003. Finally, column (15) is computed from quantities already obtained.

TABLE 11

$-2r = +1{\cdot}2$, $\quad 1/2(1-r^2) = 0{\cdot}78125$, $\quad 1/2\pi\sqrt{(1-r^2)} = 0{\cdot}19894$

(11) q	(12) $q\left(p + \frac{c}{1-c}\right)$	(13) ϕ	(14) ψ	(15) $(q^* - q)\psi/q\left(p + \frac{c}{1-c}\right)$
0·7497	0·2029	3·8021	0·004440	−0·00256
0·5938	0·2533	1·8736	0·03055	−0·00762
0·4035	0·2489	0·6605	0·1028	−0·02338
0·2037	0·1664	0·0517	0·1889	−0·11545
0·0625	0·0599	0·1879	0·1649	−0·00358
0·0099	0·0100	1·2204	0·05871	+0·18141
0·0011	0·0011	2·8500	0·01151	−0·01151
0·0001	0·0001	4·8477	0·001561	−0·00156

The second stage is completed by finding

$$G = Sn(q^* - q)\psi/q\left(p + \frac{c}{1-c}\right),$$

here equal to 0·7951. Generally, if G is positive, the value of r which makes it zero is algebraically larger than the trial value, and reversely. Therefore we try next a value above $r=-0{\cdot}60$, say $-0{\cdot}55$, and the second stage of computation is repeated with the new value of r, giving $G=-3{\cdot}0043$. Evidently the maximum likelihood estimate lies between $-0{\cdot}60$ and $-0{\cdot}55$, much closer to $-0{\cdot}60$, so we require to narrow the mesh of r in the neighbourhood of $-0{\cdot}60$. For this purpose, column (11) is computed with $r=-0{\cdot}65$ and Table 12, containing values of q, constructed.

TABLE 12

	Dose			
r	2·47	3·20	4·10	5·34
−0·55	0·7500 $^{-3}$	0·5954 $^{-16}$	0·4086 $^{-51}$	0·2129 $^{-92}$
−0·60	0·7497 $^{+2}$	0·5938 $^{+2}$	0·4035 ^{0}	0·2037 $^{-5}$
−0·65	0·7496 $^{-1}$	0·5924 $^{-14}$	0·3984 $^{-51}$	0·1940 $^{-97}$

	Dose			
r	7·11	9·60	12·45	15·65
−0·55	0·0708 $^{-83}$	0·0130 $^{-31}$	0·0018 $^{-7}$	0·0002 $^{-1}$
−0·60	0·0625 $^{+1}$	0·0099 $^{+3}$	0·0011 $^{+2}$	0·0001 ^{0}
−0·65	0·0543 $^{-82}$	0·0071 $^{-28}$	0·0006 $^{-5}$	0 $^{-1}$

The second stage is then repeated with $r=-0{\cdot}58$, $-0{\cdot}59$ and $-0{\cdot}61$, column (11) being computed in each case from this auxiliary table of q. The results so far can be summarized in a table of G:

r	G
−0·58	−0·828 $^{+776}$
−0·59	**−0·052** $^{+847}$ **+71** $^{+46}$ −25
−0·60	**+0·795** $^{+893}$
−0·61	+1·688

Inverse interpolation to find the value of r making G zero is performed using the numbers in black by the first method described on p. 935 of *Interpolation and Allied Tables* (H.M. Stationery Office, 1942): $G=0$ when $r=-0{\cdot}591$.

Similarly, we can compute values of

$$V=Sn\beta^2/q\left(p+\frac{c}{1-c}\right)$$

for each value of r, and obtain

r	V
−0·58	0·01713 $^{-30}$
−0·59	0·01683 $^{-22}$ **+8**
−0·60	**0·01661**

so $V(r)=V(-0{\cdot}591)=0{\cdot}01680$.

Table 13

$\pm r$	$\frac{1}{2(1-r^2)}$	$\frac{1}{2\pi\sqrt{(1-r^2)}}$	$\pm r$	$\frac{1}{2(1-r^2)}$	$\frac{1}{2\pi\sqrt{(1-r^2)}}$
0·00	0·50000	0·15915+	0·50	0·66667	0·18378
0·01	0·50005+	0·15916	0·51	0·67577	0·18503
0·02	0·50020	0·15919	0·52	0·68531	0·18633
0·03	0·50045+	0·15923	0·53	0·69531	0·18768
0·04	0·50080	0·15928	0·54	0·70582	0·18910
0·05	0·50125+	0·15935+	0·55	0·71685−	0·19057
0·06	0·50181	0·15944	0·56	0·72844	0·19210
0·07	0·50246	0·15955−	0·57	0·74063	0·19370
0·08	0·50322	0·15967	0·58	0·75347	0·19537
0·09	0·50408	0·15980	0·59	0·76699	0·19712
0·10	0·50505+	0·15996	0·60	0·78125	0·19894
0·11	0·50612	0·16013	0·61	0·79631	0·20085+
0·12	0·50731	0·16031	0·62	0·81222	0·20285−
0·13	0·50860	0·16052	0·63	0·82905−	0·20494
0·14	0·51000	0·16074	0·64	0·84688	0·20713
0·15	0·51151	0·16098	0·65	0·86580	0·20943
0·16	0·51314	0·16123	0·66	0·88590	0·21185−
0·17	0·51488	0·16151	0·67	0·90728	0·21439
0·18	0·51674	0·16180	0·68	0·93006	0·21707
0·19	0·51873	0·16211	0·69	0·95438	0·21989
0·20	0·52083	0·16244	0·70	0·98039	0·22286
0·21	0·52307	0·16278	0·71	1·00827	0·22601
0·22	0·52543	0·16315+	0·72	1·03821	0·22934
0·23	0·52793	0·16354	0·73	1·07043	0·23287
0·24	0·53056	0·16395−	0·74	1·10522	0·23662
0·25	0·53333	0·16437	0·75	1·14286	0·24062
0·26	0·53625+	0·16482	0·76	1·18371	0·24488
0·27	0·53932	0·16529	0·77	1·22820	0·24944
0·28	0·54253	0·16579	0·78	1·27681	0·25433
0·29	0·54591	0·16630	0·79	1·33014	0·25959
0·30	0·54945+	0·16684	0·80	1·38889	0·26526
0·31	0·55316	0·16740	0·81	1·45391	0·27140
0·32	0·55704	0·16799	0·82	1·52625+	0·27807
0·33	0·56110	0·16860	0·83	1·60720	0·28534
0·34	0·56536	0·16924	0·84	1·69837	0·29333
0·35	0·56980	0·16990	0·85	1·80180	0·30213
0·36	0·57445−	0·17059	0·86	1·92012	0·31189
0·37	0·57931	0·17131	0·87	2·05677	0·32280
0·38	0·58439	0·17206	0·88	2·21631	0·33508
0·39	0·58969	0·17284	0·89	2·40500+	0·34905+
0·40	0·59524	0·17365+	0·90	2·63158	0·36513
0·41	0·60103	0·17450	0·91	2·90867	0·38387
0·42	0·60709	0·17537	0·92	3·25521	0·40609
0·43	0·61342	0·17628	0·93	3·70096	0·43300
0·44	0·62004	0·17723	0·94	4·29553	0·46649
0·45	0·62696	0·17822	0·95	5·12821	0·50970
0·46	0·63420	0·17924	0·96	6·37755+	0·56841
0·47	0·64177	0·18031	0·97	8·46024	0·65468
0·48	0·64969	0·18142	0·98	12·62626	0·79978
0·49	0·65798	0·18258	0·99	25·12563	1·12822

Note. 0·50125+ means 0·5013 to four decimal places; 0·71685− means 0·7168 to four decimal places. 0·78125 with no additional sign here means 0·78125 exactly.

Discussion

As is well known, correcting for natural mortality in the ordinary way, i.e. by Abbott's formula, assumes that the treatment and the natural factors kill the insects in different physiological ways. It is, perhaps, less widely appreciated that a further assumption is made—namely, that the individual resistances to the treatment and to the natural mortality are uncorrelated. If these resistances were completely and positively correlated, no correction for natural mortality would be necessary so long as the mortalities in the treated batches were higher than in the untreated; this is shown by equations (1) and (2). If, on the other hand, these resistances were completely and negatively correlated (admittedly an unlikely contingency), the mortalities less than 100 % in the treated batches would be corrected simply by subtracting from them the natural mortality. This is shown by equations (4) and (5).

Pairs of insecticides to which the individual resistances are strongly and negatively correlated are much to be desired. As Plackett & Hewlett (1948) have pointed out, such pairs of insecticides in mixtures would control insects effectively and economically; but in fact they would do more. They would also tend to prevent the development of resistant strains of insects, as has happened in the past after certain insecticides have been extensively used. So long as every insect treated received a dose of both insecticides in the mixture, the insects most resistant to either insecticide would be killed: only those of intermediate resistance would tend to survive, and any survivors could be expected to give rise to descendants also of intermediate resistance. These considerations apply to other fields. Pathogenic organisms tend to develop strains resistant to chemotherapeutics such as penicillin and the sulphonamides, and this might be prevented in the same way. In fact, resistant strains of any noxious organism might be avoided by the use of suitable pairs of substances.

The reaction of a resistant strain may indicate whether two poisons act on different physiological systems. A strain of insect, for example, unusually resistant to an insecticide, presumably appears as the result of the repeated selection of resistant individuals in successive generations, i.e. the repeated breeding from the survivors in the populations treated. If two poisons, A and B, act similarly, i.e. on the same physiological system, the resistances to A and to B are positively correlated (see Bliss, 1939). Hence if the use of insecticide A produces a strain of insect resistant to A, this resistant strain would also be resistant to insecticide B. If, on the other hand, the two insecticides acted respectively on different systems, a strain resistant to A need not be resistant to B also; although it could be. Thus if a strain resistant to A is not resistant to B, A and B probably attack different systems; but if the strain is resistant to both A and B, A and B may or may not attack different systems —there is no evidence either way. Keiding & van Deurs (1949) encountered strains of housefly more resistant than the normal to D.D.T. and compounds of similar chemical structure; but these strains were not more resistant than the normal to

substances of dissimilar structure, viz. B.H.C., chlordane and toxaphene. This indicates that the system acted upon by D.D.T. is different from that acted upon by B.H.C., chlordane, or toxaphene. It seems very likely that D.D.T. and the allied compounds acted similarly. That Blickle, Capelle & Morse (1948) found that a strain of housefly resistant to B.H.C. was also resistant to D.D.T. does not contradict the hypothesis that these two insecticides act on different systems.

Analysis of our data yielded some peculiar conclusions (see Table 7). Pyrethrins and D.D.T. could have acted independently both as films and direct sprays: if so, the correlation in resistance was positive for the films, but negative for the direct sprays. D.D.T. and B.H.C. could have acted independently as films, but not as direct sprays. It is true that the method of application might alter the correlation in resistance or even the whole mode of joint action, but this seems unlikely. It is possible, however, that special effects interfered with the simple joint action of the insecticides. Consider the action of the films of pyrethrins and D.D.T. The pyrethrins alone knocked the beetles down very shortly after they were confined on the films, long before any died: the D.D.T. alone did not knock the beetles down until shortly before they died. Most of the D.D.T. therefore probably penetrated into the beetles through their feet. The beetles confined on the films of pyrethrins and D.D.T. together were knocked down rapidly, just as they would have been on pyrethrins alone. Much of the dose of D.D.T. could then have penetrated through their dorsa. Thus the presence of the pyrethrins probably altered the conditions of dosage for the D.D.T. Although the circumstances and mechanism were different, it has been shown that pyrethrins increased the dose of D.D.T. accumulated by mosquitoes exposed to an insecticidal mist (David & Bracey, 1946).

The films of B.H.C. knocked the beetles down only slightly more quickly than those of D.D.T. The presence of B.H.C. thus scarcely affected the conditions of dosage for the D.D.T., and vice versa; and it was found that the D.D.T. and B.H.C. could have acted independently. Although superficially the conditions of dosage for direct sprays of D.D.T. and B.H.C. alone and together appeared the same, the tests showed, unexpectedly, that as a direct spray these two insecticides did not act independently. No explanation for this is apparent.

Both the film and direct spray tests indicated that pyrethrins and B.H.C. did not act independently. Here again, however, the pyrethrins may have altered the conditions of dosage for the other insecticide. It has been shown that the films of B.H.C. alone in P 31 kill *Tribolium castaneum* beetles at least partly, and probably largely, by fumigant action (Hewlett, unpublished). Hewlett (1947) has shown that *T. castaneum* beetles directly sprayed with pyrethrins lose weight rapidly; and has also shown (unpublished) that beetles lose weight rapidly when confined on pyrethrum films, but not on D.D.T. or B.H.C. films. The loss of weight due to the pyrethrins is so rapid that it almost certainly represents a loss of water. The water is probably lost because the pyrethrins cause the spiracles to open, and opening of the spiracles would presumably favour the entry of B.H.C. vapour. The presence of

the pyrethrins may therefore increase the dose of B.H.C. Even if the water was not lost through the spiracles, general conditions inducing loss of moisture from the beetles might well favour the entry of B.H.C.

Possible interactions such as those just described are in no way incompatible with the essential condition of independent action—namely that an organism responds by the failure of one or other or both of two different physiological systems respectively attacked by the two poisons. The systems, of course, need not be systems in a gross anatomical sense, such as the digestive or excretory systems. They are likely to be enzyme systems, interference with which triggers changes producing the response.

Perhaps another, hypothetical, example would make clearer how insecticides could interact and yet cause different systems to fail. Suppose that pyrethrins kill an insect by attacking the nervous system and that a chemically inert abrasive dust kills it by causing it to lose moisture. The two insecticides, it may be supposed, when applied separately kill the insect by causing different systems to fail. However, if the two were applied together, the pyrethrins being sorbed on the dust, abrasion of the cuticle by the dust might assist the pyrethrins to penetrate, lowering the individual minimum lethal dose of the latter; and yet the two insecticides acting together could kill by causing either or both of two systems to fail.

In the interactions mentioned, one insecticide has, it has been supposed, affected the dosage of the other. As another possibility, one insecticide might indirectly upset the reaction of the system upon which the other acted directly.

These considerations indicate that independent action should be redefined, to appear as a special case of a type of joint action termed *dissimilar*. If two poisons act dissimilarly, they act respectively on different systems to produce the response. Dissimilar action is subdivided into *independent* and *dependent* action. If poisons act independently they do not interact, if dependently they do interact. Dissimilar action and its two subdivisions are formally defined below. The definition allows for the possibility that two poisons might each act on a number of systems, and might therefore act dissimilarly in producing one response but not dissimilarly in producing another. It is worded to be as general as possible, to apply, not only to poisons, but to any drug, i.e. any substance producing a biological response.

(i) *Dissimilar joint action.* If two drugs, A and B, when administered separately to an organism elicit a common response, and if when administered jointly elicit the response by causing separate and distinct physiological systems, (A) and (B) respectively, to react or fail, the joint action of A and B is dissimilar with respect to that response.

(i*a*) *Independent joint action.* The two drugs act independently if in addition to (i), A alters neither the amount of B acting on (B) nor the reaction of (B) to B; and reversely with A and B interchanged.

(i*b*) *Dependent joint action.* The two drugs act dependently if, in addition to (i), A modifies the amount of B acting on (B) and/or the reaction of (B) to B, and/or B modifies the amount of A acting on (A) and/or the reaction of (A) to A.

It is assumed of course that A and B do not react together chemically. It may be felt that the hypothesis of dependent action is more plausible than that of independent action, as an organism is a co-ordinated whole. Dependent action certainly seems more likely where the response involves profound physiological changes such as death. Where, however, the physiological changes are shallow, independent action becomes less improbable. We are investigating the hypothesis of dependent action to see whether our data are consistent with it.

Unlike Bliss's (1939) definition of independent action, the definition just given does not mention correlation in resistance. This correlation is omitted because it is to be regarded, not as a part of dissimilar action, but as a further factor that must be taken into account in predicting the response to a mixture of drugs.

The problem we have considered is part of a wider one—how to relate the level of quantal response to the individual doses of two drugs jointly administered. One approach, which we have adopted, is to set up a hypothesis of the mode of joint action, and to derive a relationship accordingly. The derivation of the relationship may be conveniently referred to as the development of the hypothesis. In our opinion certain principles should guide the setting up of the hypothesis and its development. The hypothesis of the mode of joint action should be conceived physiologically. Moreover, its quantitative implications alone need not be sufficient for its complete development. Take, for example, the hypothesis of independent action just stated. It follows from sections (i) and (i*a*) that an organism will respond if it receives an effective dose of A, or of B, or effective doses of both A and B. This is not sufficient, however, to relate the mixture response to the doses, for the response is also influenced by correlation in resistance. It is then assumed that this correlation can properly be measured by a product moment coefficient, and so equation (9) becomes the most satisfactory of a number of alternatives for giving the mixture response. Distinguishing clearly the physiological hypothesis, the deductions from it, and the further assumptions, helps the development of the hypothesis to proceed in an orderly, logical manner. One of us (P.S.H.) has developed a hypothesis of similar action in this way, and the result is different from Finney's (1942) treatment: the latter appears as a special case, albeit an important one.

Statistical prediction of the responses due to mixtures of drugs on the assumption of independent or similar action can be useful in two ways. First, it may indicate how drugs act together. If statistical prediction is used for this purpose, dosage interaction, such as probably occurred in the laboratory tests described earlier in this paper, should be eliminated if possible. Secondly, it can provide standards by which to decide whether drugs can be advantageously combined by virtue of some special interaction. Such standards are badly needed. All too often are insecticides, for example, claimed to act 'synergistically', without any attempt being made to show how toxic they might be did they not interact.

In conclusion, we urge that physiological investigation into the joint action of insecticides would be valuable. It would doubtless reveal shortcomings in the current hypotheses of joint action, and suggest other hypotheses. At the same time,

if carried far enough, it would set the subject on a firmer foundation, and enable the quantal responses produced by mixtures of insecticides to be predicted with greater confidence—or at any rate less uncertainty.

This work has been carried out as part of the programme of research of the Pest Infestation Laboratory, and this account is published by permission of the Department of Scientific and Industrial Research.

We are indebted to Mr G. S. James of the Mathematics Division, National Physical Laboratory, for completing the computation of the regression lines; to Dr C. W. Jones for refining the method of estimating r; and to Miss O. E. Vanes for doing the appropriate calculations. Miss B. Clayton assisted in the experimental work.

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OBITUARY NOTICE

Dr A. D. IMMS

Augustus Daniel Imms was one of the original members of the Association, and for many years he took a lively interest in its affairs serving it in the capacity of President, 1930–1; Vice-President, 1924–7; Treasurer, 1921–31; and on the Council, 1914–20 and 1933–5.

Those who had the privilege of knowing him will long remember with respect the tall, unassuming, somewhat foreboding figure; the austere countenance, lit up less frequently than one could desire by a genial smile and obvious enjoyment of things interesting and good; the reserve, hard to penetrate, which covered an innate shyness or even nervousness due no doubt in part to the extremely high standard he set himself; and the feeling, unfortunately often appreciated more fully after the event, of security in his judgement whether it was appraisal or adverse criticism. Those who had the good fortune to see him working could not help being impressed by his infinite care and patience to obtain accuracy over the smallest detail, though perhaps the standard observed was frightening and even discouraging at first.

Born on 24 August 1880 at Moseley, Worcestershire, Imms went to St Edmunds High School, Birmingham, when W. B. Grove the mycologist was headmaster. Severe attacks of asthma, while preventing the more usual boyish occupations, provided the opportunity for his friendship with C. F. Olney of the Northampton Natural History Society and the awakening of his lasting interest in insects. It was at this stage that Imms's encyclopaedic knowledge of all things entomological started to accumulate. His avidity for information at this period is not difficult to imagine—it never ceased, though much later the rapid development of insect physiology was to bring home the limitations that an individual must always endure.

Attendance at the Mason University College, Birmingham, resulted in graduation as B.Sc. London with second-class honours in zoology in 1903. The next two years were spent at Birmingham working with T. B. Bridge on fishes. In 1905 the award of an 1851 Exhibition Scholarship led Imms to Christ's College, Cambridge, where A. E. Shipley was his tutor. Here he was in contact with such men as G. H. F. Nuttall, F. Jenkinson, G. H. Verrall, J. E. Collin, C. G. Lamb and F. Muir and had among his contemporaries, J. C. F. Fryer and H. Scott. In the days before the Ph.D. at Cambridge Imms graduated B.A. by research on the anatomy of the immature stages of *Anopheles maculipennis* in 1907. The same year he also obtained the Darwin Prize of Christ's College and the D.Sc. Birmingham.

Then through lack of vacant posts in entomology, Dr Imms was forced to leave this field for a short while and was appointed to the Professorship of Biology at the University of Allahabad. Thus began a succession of appointments that caused Imms, the builder of departments, to emerge. In 1911 he became Government of India Forest Entomologist with headquarters at Dehra Dun. Here new laboratories had to be designed. But there were opportunities to travel and to study insects. It was during these days that the broadening of outlook, in his case in biology, morphology and the wide problems of agricultural and economic zoology, so desirable for most men, was obtained. It was here too in India that the second period of original work took place resulting in studies on a Nemopterid, Indian Collembola and an Embiid.

In 1913 Imms was appointed to the Readership in Agricultural Entomology at Manchester. His rejection by recruiting boards reminded him of the ill-health that caused his departure from India, but his desire to be useful found relief in his acting as additional crop pest inspector and reporter for the Board of Agriculture.

The next step was to Rothamsted Experimental Station in 1918 to be the first Chief Entomologist and again to found a department. Instead of research this period saw the publication in 1925 of *A General Text-book of Entomology*. The appearance of this remarkable

work followed by *Recent Advances in Entomology* (1930), *Social Insects* (1931) and somewhat later *Outlines of Entomology* (1942) revealed Imms's great knowledge, accuracy of detail, morphological insight, critical acumen and gift of writing clearly. It was also during the years in Harpenden that his visit to the U.S.A. took place and his interest in the biological control of insects and weeds became apparent. In 1929 the Royal Society elected him to its Fellowship.

In 1931 Imms went to the newly established Readership in Entomology in the Department of Zoology, Cambridge. Once more a new sub-department needed a builder, but having accomplished this Imms entered his third period of original research. This time the attraction was the ancestry of insects, the antennal musculature in insects and other arthropods, the antennal growth processes, the constitution of the maxillae and labium in Mecoptera and Diptera and finally that curious insect *Braula caeca*. Once more the wide outlook combined with accuracy of detail and clarity of expression was apparent.

In 1936–7 Imms was President of the Royal Entomological Society of London; he was a Vice-President four times and on its Council twice. In 1944 the Society elected him Special Life Member. For many years a member of the Linnæan Society of London he served twice on its Council. Dr Imms was recognized abroad by being elected a Corresponding Member of the Agricultural Academy of France and of the Entomological Society of Finland and by being made an honorary member of the Entomological Societies of Holland and India.

The second World War revealed a new Imms in his successful work as bursar and steward of Downing College of which he was elected a Fellow in 1940. He retired in 1945 and his services were recognized by the honour of being elected to an Honorary Fellowship.

But retirement in Devon did not mean the end of his activities. There was more time for studying insects in the field, for gardening, walking and motoring; local affairs became interesting; and his books needed further editions. He was elected a Foreign Member of the American Academy of Arts and Sciences in 1947. Finally appeared *Insect Natural History* (1947) which reveals his intense love and great knowledge of insects in a manner which surprised some of his fellow workers who did not expect this from an acknowledged morphologist, teacher, text-book writer and builder of departments.

Imms was not free from worries for, besides his own ill-health both in his youth and later years, his wife, whose ever-cheerful Irish spirits were so complemental to his own somewhat quiet nature, had the great misfortune to suffer two paralytic strokes which left her a permanent invalid. Mrs Imms and their two daughters survived him, who had in the last few years to fight courageously against more and more pain. His letters at this time seemed to indicate a calm realization of what was inevitable. The end came on 3 April 1949.

I am indebted for some of the factual matter contained in the above to *Obituary Notices of Fellows of the Royal Society* (November 1949), in which Dr Imms's chief works are enumerated.

H. F. Barnes

REVIEW

A List of Abbreviations of the Titles of Biological Journals. Issued by the Biological Council. Pp. iv+26. London: H. K. Lewis and Co. 1949. *2s. 6d.*

Many scientific journals, including the *Annals of Applied Biology*, request their contributors, in citing references, to use the abbreviations given in the *World List of Scientific Periodicals*. For authors who do not have ready access to a copy of the *World List*, the Biological Council has issued this booklet, giving the standardized abbreviations for the titles of nearly 800 of the more widely used biological journals. The selection has been made by permission of the *World List*, whose author, Mr F. L. Kent, contributes a foreword, including a brief review of the progress achieved in securing international agreement in this field.

It is hoped that the low cost of the Biological Council's booklet will ensure its wide use, leading eventually to the production of authors' reference lists that are impeccable, even in the typescript stage.

R. W. MARSH

PLANT PESTS AND DISEASES COMMITTEE REPORT ON SPRAY DAMAGE CONFERENCE

The effects of spray damage to crops are known only too well to the applied chemists, entomologists and mycologists who work in close association with agriculture and horticulture. The *causes* of spray damage are *not* known, and it has generally been agreed that the major contribution towards solving this complex problem must be provided by plant physiologists.

In an effort to stimulate the interest of plant physiologists in this subject, an informal conference was held in January 1949 at Imperial College, London, to discuss spray damage with special reference to top fruit. The conference was attended by about thirty scientific workers including plant physiologists, chemists, entomologists and mycologists who had had considerable experience in this field.

The problem was first outlined by four speakers: Dr H. Martin, Long Ashton, Mr M. H. Moore, East Malling, Prof. A. E. Muskett, Northern Ireland and Dr W. A. R. Dillon Weston, Cambridge. They covered the various aspects of damage caused by the application in the dormant or in the growing season by oils, sulphur compounds, copper compounds, mercurials, arsenicals and combinations of these (and here it is important to realize that new insecticides and fungicides are continually being brought into use) and the influences of methods of application, varieties, nutrition and locality.

From the stimulating discussion which took place it was obvious that the plant physiologists were unaware of the vast amount of spray damage that occurred annually in the country. This fact is not surprising when one considers that only a very small proportion of the cases are placed on record in scientific papers, and these are only observations made by entomologists and mycologists when carrying out field trials for the control of some specific insect or fungus.

There emerged from the discussion a number of specific problems, on which information is required:

(1) The physiological processes underlying abscission of both leaves and fruits following the application of sprays.

(2) The specific influence of auxin modifications on dormancy and abscission (this may be covered in (1)).

(3) The effect of oil films on general metabolism of buds. This subject could be enlarged if desired to include the phytotoxic action of oils used in post-dormant washes.

(4) The underlying causes of sensitivity in varieties of fruit. Here sulphur shyness, which occurs so markedly in a group of varieties of the European gooseberry (*Ribes grossularia* L.), would form a useful subject for investigation, although the problem is by no means restricted to soft fruit.

(5) The underlying causes of sensitivity of leaves at different stages of development to damage by sprays. It may be possible to link this with (4).

(6) The entry of inorganic and organic molecules into the plant with emphasis on absorption into leaf tissue:

- (*a*) The effect of the chemical composition of the material as applied to the plant and the identification of the specific component(s) or compounds responsible for damage.
- (*b*) The mode of entry of the toxic agent into the plant.
- (*c*) The detailed study of the damage to the plant anatomically or physiologically.

(7) The shade effect of spray deposits on photosynthesis and other functions.

It was generally agreed at the meeting that it was desirable to bring these specific fundamental physiological problems to the notice of heads of Plant Physiology Departments where it was hoped the opportunity might arise from time to time of directing the researches of post-graduate workers towards solving some of these problems.

REPORT OF THE COUNCIL OF THE ASSOCIATION OF APPLIED BIOLOGISTS FOR THE YEAR 1949

The Officers and Council of the Association were as follows:

President: G. Fox Wilson, N.D.H., F.R.E.S.
Vice-Presidents: W. C. Moore, M.A.; J. W. Evans, M.A., Sc.D.
Hon. Treasurer: H. F. Barnes, M.A., Ph.D.
Hon. Secretary: R. K. S. Wood, A.R.C.Sc., Ph.D.
Hon. Asst. Secretary: J. R. Busvine, D.Sc.
Hon. Editor of the *Annals of Applied Biology:* R. W. Marsh, M.A.
Hon. Asst. Editor: I. Thomas, M.Sc., Ph.D.

Council: J. R. Booer, Ph.D.; C. Ellenby, D.Sc.; J. W. Evans, Sc.D.; S. D. Garrett, Sc.D.; H. H. Glasscock, M.Sc.; H. C. Gough, Ph.D.; F. H. Jacob, M.Sc.; D. Price Jones, Ph.D.; W. G. Keyworth, Ph.D.; D. Snow, Ph.D.; R. L. Wain, D.Sc.; Mrs M. A. Watson, Ph.D.

Membership of the Association continued to increase. During the year fifty members were elected. Thirteen resignations were accepted and on 31 December 1949 membership stood at 558, a new high figure.

The Council has to announce with deep regret the deaths during the year of the following members: Prof. F. Sylvestri and Dr A. D. Hopkins, both Honorary Members and distinguished entomologists; Mr H. Stone, one of the oldest members and first Honorary Treasurer; Dr A. D. Imms, who had served as Honorary Treasurer and as President of the Association.

The Council were pleased to congratulate Sir William Ogg and Sir Ronald Hatton on the Honour of Knighthood bestowed upon them.

Six meetings were held in London with an average attendance of fifty-four members and thirty-six visitors. The following papers were read:

18 February. Presidential Address. Mr W. C. Moore: The significance of plant diseases in Great Britain.

16 March. Symposium. Growth-promoting substances in agriculture and horticulture. Prof. F. G. Gregory: Introduction. Miss D. Osborne: Attempts to produce parthenocarpic pears with growth substances. M. C. Vyvyan: The use of growth substances to control the shedding of fruit. R. L. Wain: Chemical aspects of growth-regulating activity. E. S. J. Hatcher: The use of growth-promoting substances in the vegetative propagation of plants. L. C. Luckwill: Fruit-drop in the apple in relation to seed-development.

6 October. Virus diseases of trees. R. S. Willison: Virus diseases of stone-fruit trees. A. F. Posnette: Virus diseases of cacao. Informal contributions by S. H. Crowdy, L. C. Luckwill and I. W. Prentice.

4 November. J. R. Busvine: Mosquito eradication campaigns in Cyprus and Sardinia (with a film). Miss C. M. Harrison: D.D.T.-resistant houseflies.

2 December. Symposium on clover. Watkin Williams: Clover breeding. J. Morgan Jones: Clover seed weevils. W. A. R. Dillon Weston: Clover sickness. T. Goodey: Stem eelworm and clover. H. H. Mann: Non-parasitic clover sickness. L. Ogilvie and Mrs H. Justham: Recent investigations on clover rot.

At the kind invitation of the Council of the Royal Horticultural Society, the Field meeting was held at Wisley on 6 May. A total of about 100 members and guests attended and were entertained to luncheon and tea by the Society. The morning was spent in the Laboratories. During the afternoon parties were conducted around the Gardens by members of the Staff.

The Summer Meeting was held at Nottingham on 9–11 June. Through the kindness of the Directors, extensive visits were paid to the factories, laboratories and Experimental Stations of Boots Pure Drug Co. Ltd. The Council wish to record their deep appreciation of the work done by Miss Wilson and her colleagues in connexion with this meeting.

Following the preparation of a report by the Plant Pests and Diseases Committee on the application of the Priority Rule in Nomenclature to the Names of Economic Plants, a Committee was formed to consider the way in which the proposals of this report would best be implemented. The following kindly agreed to serve on this Committee: Mr Hemming, Dr Findlay, Dr Ramsbottom, Colonel Stern and Dr Wiltshire. A report, from which Dr Wiltshire dissented, was prepared, agreed by the Council and the Plant Pests and Diseases Committee and submitted for consideration to the Nomenclature Section of the 7th International Botanical Congress, 1950.

The Council wish once again to thank the authorities of Imperial College for their kindness in providing lecture theatres for meetings, and for the free use which has been made of their catering facilities.

HON. EDITOR'S REPORT FOR 1949

Vol. **36** of *The Annals of Applied Biology* consisted of pp. xi. + 572 and 13 Plates, a decrease of 80 pages and an increase of 6 Plates on vol. **35**. Including papers published in *Proceedings* vol. **36** contained 51 communications, 44 being by members of the Association. The subjects may roughly be classified as follows: general applied botany, 8; mycology and fungus diseases, 6; viruses and virus diseases, 10; entomology and insect pests, 8; insecticides and fungicides, 15; statistical methods, 4.

The printing order for 1949 remained at 1450 copies.

As a result of the measures detailed in the Editor's report for 1948, a considerable reduction in publication delays was achieved in 1949. The dates of issue of the four parts were: No. 1, 28 March; No. 2, 7 July; No. 3, 19 October; No. 4, 1 February.

The Editor assisted in the preparation, for the Biological Council, of a selected list of the standardized abbreviations of titles of biological journals. This was issued as a booklet in December 1949, with the aim of providing guidance to authors in the correct citation of references.

The Editors express their sincere thanks to the members of the Publications Committee, whose advice plays a major part in the maintenance of the high reputation of the *Annals*. During the greater part of the year under review, the Editor was overseas, and he, together with the Association as a whole, is deeply indebted to Dr Thomas, who shouldered almost the entire burden of the editorial responsibilities during this period.

PLANT PESTS AND DISEASES COMMITTEE, REPORT FOR 1949

Three meetings of the Committee have been held during the year on 18 February, 6 October and 20 January. H. E. Croxall, N. G. Morgan and R. S. Pitcher were appointed by Council to fill the vacancies caused by retirement under the rules of C. J. Hickman, F. J. W. Jones and R. W. Sidwell. At the first meeting P. H. Gregory was elected Chairman and S. H. Crowdy, Secretary.

The Sub-Committee formed in collaboration with the Ministry of Agriculture has continued its compilation of the list of common names and scientific names of insects and other pests: those on Acarina and Anoplura have been completed.

A revision course in Plant Nematology was held during July at the School of Agriculture, Cambridge, by kind permission of Prof. Sir Frank Engledow, and the Committee would like to congratulate Dr T. Goodey and Mr F. G. W. Jones on the success of the course and to express their appreciation of the trouble which they have taken over it.

A suggestion that some alteration in the rules of Botanical Nomenclature should be made, in order to allow the retention of specific names in cases where change would cause unnecessary confusion, was transmitted to Council. A report prepared by a Sub-Committee of Council was approved and a request made that Council should take appropriate action.

The Sub-Committee which was appointed to consider existing systems of Classification and Nomenclature of Plant Viruses has submitted a report which is to be published in due course.

A Sub-Committee has been appointed to prepare a list of definitions of terms used in Economic Entomology.

REPORT OF THE HONORARY TREASURER FOR THE YEAR ENDED 31 DECEMBER 1949

During the year ended 31 December 1949, the amount received for subscriptions and entrance fees, including arrears, was £655. 2*s*. 6*d*., an increase of £6. 12*s*. 6*d*. This comparative decrease is largely accounted for by more members being in arrears at the end of the year. Members are reminded that under Rule 7, they can be struck off the list of members as soon as they are one year or more in arrears in the payment of their subscription.

Income for the sale of the current volume of the *Annals of Applied Biology* amounted to £1589, an increase of £116. 2*s*. 0*d*. over the sale of the *Annals* in 1948. The cost of production was £2100. 17*s*. 0*d*., an increase of £5. 3*s*. 5*d*. The income from the sale of back volumes and parts was £274. 16*s*. 0*d*., a decrease of £2. 7*s*. 6*d*., while the sale of reprints showed an increase of £23. 12*s*. 8*d*.

Income for the year showed an excess of £537. 19*s*. 4*d*. over the expenditure, as against £379. 0*s*. 4*d*. in the previous year. This is accounted for by the much reduced deficit on the *Annals*. It will be noted that £20. 2*s*. 3*d*. has been spent on refreshments—this is for teas after meetings, which the Council consider to be an important factor in the usefulness of the Association in promoting friendship amongst its members.

The Association received a grant of £150 from the Royal Society in aid of publication. This grant is gratefully acknowledged. In addition two bequests of £105 and £100 have been received from the late Mr H. Stone and the late Dr G. H. Pethybridge respectively.

During the year, 1000 National Savings Certificates (8th issue) costing £500 were purchased as well as £400 worth of 2½% Defence Bonds. The accumulated interest on the holding of National Savings Certificates amounted to £448. 5*s*. 1*d*. at 31 December 1949.

The net assets of the Association at the end of the year amounted to £3041. 6*s*. 6*d*., an improvement of £892. 19*s*. 4*d*. as compared with a year ago. All available surplus funds are invested in the Post Office (£1260. 1*s*. 9*d*.), National Savings Certificates (£1338. 6*s*. 1*d*.) and 2½% Defence Bonds (£400). The estimated value of the stock of the *Annals of Applied Biology* is £153. 3*s*. 0*d*.

While the position of the Association is at present satisfactory the following points should be remembered. The abnormally large annual increase in membership of the immediate post-war years may now be expected to assume a more normal figure. The sale of *Annals* to non-members has for the fourth year in succession exceeded the number supplied to members. The figure for 1949 was 695 compared with 564 members. The sale of back volumes and parts, while still an extremely valuable source of income, may be expected to have reached its peak. On the other hand, the sale of reprints is assuming pre-war figures. Finally, the Cambridge University Press has given notice that from the end of the current volume (36) a new schedule of charges incorporating all recent advances in cost will be prepared. This will inevitably result in an advance in charges for the printing of the *Annals*.

THE ASSOCIATION OF APPLIED BIOLOGISTS

Dr. *ANNALS OF APPLIED BIOLOGY*, INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 DECEMBER 1949 *Cr.*

Expenditure	£	s.	d.	Income	£	s.	d.
To Estimated Value of Stock at 1 January 1949	117	5	0	By Sales—Current Volume	1589	0	0
To Cambridge University Press	2100	17	0	By Sales—Back Volumes, Parts and Sets	274	16	0
				By Sales of Reprints and Advertisements	148	11	5
				By Estimated Value of Stock, 31 December 1949	153	3	0
				By Balance carried down	52	11	7
	£2218	2	0		£2218	2	0

Dr. GENERAL INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 DECEMBER 1949 *Cr.*

Expenditure	£	s.	d.	Income	£	s.	d.	£	s.	d.
To *Annals of Applied Biology* balance brought down	52	11	7	By Members' Subscriptions:						
To Printing and Stationery	32	4	10	Arrears	9	16	0			
To Postage and Cheque stamps	25	17	6	Entrance Fees	31	11	6			
To Subscription—Parliamentary Science Committee	7	7	0	Current	613	15	0			
To Donation—The Biological Council	5	0	0					655	2	6
To Sundry Out-of-Pocket expenses of Editors, Secretaries and Treasurer	17	10	11	By Sundry Income—R.H.S. visit				10	15	0
To Refreshments	20	2	3	By Net Receipts—*List of Common Names*, Pt. 1				5	4	9
To Audit Fee	5	5	0	By Interest Receivable:						
To Visit to R.H.S. Laboratories, Wisley	13	7	0	National Savings Certificates	15	8	8			
To Balance, being Excess of Income over Expenditure for the year	537	19	4	Post Office Savings Bank	30	14	6			
								46	3	2
	£717	5	5					£717	5	5

BALANCE SHEET, 31 DECEMBER 1949

Liabilities and Surplus	£	s.	d.	£	s.	d.	Assets	£	s.	d.	£	s.	d.
Sundry Creditors:							Cash:						
Cambridge University Press	198	9	7				Westminster Bank: Current Account	309	14	5			
Audit Fee	5	5	0				Post Office Savings Bank Account	1260	1	9			
Sundry Expenses	12	13	2								1569	16	2
				216	7	9	1500 National Savings Certificates (Cost £890. 1s. 0d.)				1338	6	1
Subscriptions and Entrance fees paid in advance				28	11	0	2½% Defence Bonds				400	0	0
Life Membership Fund Reserve				175	0	0	Stocks of *Annals of Applied Biology* at estimated value				153	3	0
Excess of Assets over Liabilities:													
As per Balance Sheet 31 December 1948	2148	7	2										
Add: Royal Society Grant	150	0	0										
Bequests (2)	205	0	0										
Balance of Income and Expenditure Account for 1949	537	19	4										
				3041	6	6							
				£3461	5	3					£3461	5	3

H. F. BARNES, *Hon. Treasurer*

I certify that the foregoing Accounts are properly drawn up in accordance with the books, vouchers and documents produced to me, and in my opinion, the Balance Sheet exhibits a true and correct view of the state of affairs of the Association according to such books, vouchers and documents.

(Signed) J. B. BENNETT *Chartered Accountant* *Auditor*

HARPENDEN, 14 *April* 1950